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THE SO-CALLED STRANDS AND SECONDARY TUMORS IN THE CROWN GALL DISEASE

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WITH PLATES XVII TO XX

So far the so-called strands and secondary tumors in neoplasia of plants have been shown to have no significance other than the possible analogy they may bear to metastases in human cancer. Smith (16) claims that *Bacterium tumefaciens* when inoculated into plant tissues produces types of growths that are analogous to sarcomas, carcinomas, and embryomas in animal and human cancer. He makes a general assertion that, while the organism he and his colleagues discovered would not produce cancer in animals or plants, he believes that animal and human cancer are the results of parasites.

The fact that no type of neoplasia in plants was analogous to carcinoma in animals was pointed out by Levin and Levine (3) and others. The sarcoma-like growth produced by inoculation with *B. tumefaciens* into young meristematic tissues of plants has been shown to be granulomatous in nature and not malignant growth in a great majority of cases, by Levin and Levine (3). That leafy shoots in the crown-gall disease are not analogous to the embryomata in animals was later pointed out by Levine (4, 10). The claim made by Smith (16) that the crown-gall organism is an intracellular parasite is denied by Robinson and Walkden (15) and independently by Riker (13, 14). While these results have come from two different laboratories, it appears, according to Smith (17), that the question is not entirely settled. Evidence recently advanced by Petri (12) shows that the Aleppo pine tumors are related to crown galls and are due to an intracellular organism, *Pseudomonas pini* (Vuill.) Petri. Atypical divisions of cells characteristic of human and animal cancer are not found in the neoplastic diseases of plants (11).

The points that are of interest for further study are those referring to secondary tumors and tumor strands. Smith (16) claims that the crown

gall sends out "roots" or "tumor strands" into the normal tissues. These extend for some distance into the healthy tissues. These strands consist of immature, actively vegetating cells. These cells are meristematic in nature and are capable of forming medullary rays, tracheids, and sieve tubes.

In animal cancer, metastases occur by lymphatic permeation and more frequently by emboli of the cancer tissue disseminated through the blood and lymph streams.

The question of lymphatic permeation of animal cancer does not appear to be settled. Handley (2), in cancer of the breast, has shown that there may be extensions of tumor cells through the lymph channels of the deep pectoral fascia to the axillary and subclavicular nodes, then through the chest and abdominal cavities, invading all organs. Handley believes this process of lymphatic permeation is the chief method by which cancer is disseminated.

Ewing (1) points out the fact that Handley's studies were made on material at autopsy and that emboli lodging at various points in the chain of lymphatics may grow in opposite directions until they meet and so fill the lymph channel. Ewing sums up by saying, "In general it appears probable that the rapidly growing epidermoid and globular carcinomas disseminate chiefly by lymphatic embolisms while long growing and recurrent tumors especially on the skin often extend by continuous permeation."

Emboli of crown-gall tissue do not seem to be possible. Permeation of crown-gall tissues by means of "roots" and "tumor strands" have been claimed to exist by Smith (16). Secondary tumors arise from these strands. The so-called secondary tumors of plants, if such occur, arise by a method which is not generally accepted even for secondary growths in animals and human beings.

Robinson and Waldken (15) were able to produce crown galls on *Chrysanthemum frutescens* and *Nicotiana affinis*, which are similar in all respects to the crown galls designated by Smith as secondary galls. These workers contend that these so-called secondary growths result from an inoculation and subsequent growth of a meristematic apex. Another type of growth resulting from inoculations of apices is the smooth gall. These galls appear as swellings on the surface of leaves distant from the point of inoculation. These authors regard these cells as the "true secondaries." They claim that continuous chains of bacteria can be traced from the point of inoculation to the smooth growths. The growths are the result of direct stimulation of the tissue rather than the result of tumor strands extending from the crown-gall tissues at the region of inoculation.

Riker (13), in studying crown-gall formation in the tomato, pea, sunflower, and Paris daisy, holds that "secondary galls" appear only in a small percentage of cases, when the inoculations are made in rapidly growing regions. He contends that the inoculations produced by puncturing the tissue release cell fluids into the intercellular spaces of rapidly elongating tissue. This intercellular fluid bears the bacteria relatively short distances in the embryonic tissue. When the tissue matures, however, a crown gall quite distant from the point of inoculation results. Riker contends that the secondary galls and tumor strands are not really secondary growths. He contends that the galls which appear on plant tissues at some distance from the point of inoculation are stimulated to development by some of the bacteria that were introduced at the original point of inoculation. In general, the results of Riker confirm the observations of Robinson and Walkden.

The question of the formation of secondary tumors in plants is of interest in so far as the claim has been made that crown gall is analogous to animal cancer. I have been studying for the past two years the mechanism of the formation of these so-called tumor strands and secondary growths in a variety of plants. I have been engaged at the same time in studying microscopic sections of various plant tissues after inoculation with *Bacteria tumefaciens* for the purpose of locating the organisms in the crown-gall tissue. I shall report on these results in a later paper. In my study of the so-called strands and secondary tumors I have attempted to answer the following questions:

1. How far apart must inoculations with the crown-gall organism be made to affect the tissue between the point of inoculation?
2. What effect has inoculation with *B. tumefaciens* on water-soaked tissues?
3. What effects have inoculations of *B. tumefaciens* on slit tissues?
4. What results from infecting long perforations in tissues with *B. tumefaciens?*
5. What kinds of plant tissues best produce the so-called strands and secondary tumors in the crown-gall disease?

DISTANCE BETWEEN POINTS OF INOCULATION

The effect of inoculating a stem or petiole with *B. tumefaciens* at a number of points at various distances apart is of interest because of the light it may throw on the mechanism of the formation of crown galls and strands in the tissue between the points of inoculation. It must be mentioned that only young tissues susceptible to the crown-gall disease have

been used for these studies. The young shoots of the rambler rose, rubber plant, the tomato, and petioles of *Ricinus* plants were used. The midveins of the young leaves of the rubber plant were also utilized. I am emphasizing the fact that apical portions of the shoots were not used. Parts of the plants that were still green and soft and which were back of the region of elongation were tried exclusively in these experiments. The inoculations with *B. tumefaciens* were made at several points ½ cm. to 3 cm. apart. The parts were labeled carefully and then examined from time to time. The inoculations were made during the first week in July, and the galls were collected and studied in October and November. The inoculated plants were removed from the soil and photographed for purposes of record.

In the rose it was found that when the shoots were inoculated with *B. tumefaciens* at two or more points ½ cm. apart, two or more distinct crown galls developed at first. These became oppressed with subsequent growth and frequently showed fusion of the tissue of the galls. In a majority of the cases the galls retained their individuality until they died (Figs. 1, 2, and 3, Pl. XVII). When the inoculations were made from 1 cm. to 3 cm. apart, independent galls were found at each point of inoculation (Fig. 4, Pl. XVII). Free-hand sections were made of the tissue between these galls, but in no case was a new gall developed either behind the point of inoculation or toward the apical portion of the shoot.

The cortical and epidermal portions of the stem frequently seemed to be thickened, but there was no evidence that any strands occurred here. The nature of the structure of these galls was described by Levin and Levine (3). A great number of these experiments were made with the same result.

Similar results were obtained when the rubber plant was inoculated with *B. tumefaciens*. While the number of rubber plants were relatively small, the results were uniform. I found no instance of development of secondary galls between the points of inoculation. In figures 5 and 6 (Pl. XVII) a leaf is shown that had been inoculated on May 27, 1922, by puncturing the midvein at intervals of ½ cm. with a needle containing *B. tumefaciens*. The photographs were taken on November 1, 1924. In these figures we find that some crown galls developed on the ventral surface, while others formed on the dorsal surface of the leaf. In some instances crown galls developed on both sides of the leaf from the same puncture. All these experiments gave the same results. In no case was a crown gall produced on these leaves other than at the points of inoculation.

A large number of tomato plants in my cultures, used in connection with other studies, showed results similar to those noted in the rose and rubber

plant. Where the distance between the points of inoculation was small, the galls seemed to fuse. When the distance between the inoculation was greater, independent crown-gall growths appeared. Hand sections were made in a number of cases of young tomato stems immediately after they were inoculated by the puncture method to determine whether the water-soaked areas induced by the injury in inoculation would fuse. It was found that by carefully puncturing the stems the water-soaked areas were confined to a very small radius around the puncture and did not fuse. However this may be, bacteria of the size of *B. tumefaciens* may still find enough fluid liberated by the injury to enable them to move into the spaces between the punctures. The galls first appeared as independent growths but later fused into one large growth. The fact that the stems used for the experiments were young, and possibly capable of elongation, may explain the fusion of these growths. In inoculations made at greater distances, from 2 to 5 cm. apart, the results were similar to those described for the rose. I have studied the petioles of over two hundred plants of *Ricinus*. The petioles were measured before they were inoculated. Inoculations were made at single points at a distance of from 1 cm. to 3 cm. apart. In petioles over 18 cm. long, separate and distinct crown galls appeared from two to four weeks after the inoculation. I was unable to find a thickening of the tissues between the crown galls or swellings arising from crown galls at points other than those inoculated. Such thickenings and swellings occurred only in such growths as I shall describe below for petioles much smaller and younger.

BACTERIUM TUMEFACIENS IN WATER-SOAKED TISSUES

The effects of inoculating water-soaked tissues for the production of strands and secondary tumors were studied.

Young shoots of the raspberry plant (var. Cuthbert) were water-soaked by gently rolling the parts to be inoculated between the finger and thumb. The length of the shoot so treated measured from 8 to 16 cm. This distance was marked off from the rest of the branch by lines made with India ink. The part of the stem directly in back of the growing region was inoculated, so that little or no elongation occurred in the region studied. The tissues were sufficiently young to respond to the inoculation. The middle of the water-soaked area of the shoot was inoculated by pricking it with a needle dipped into a culture of *B. tumefaciens*. After the inoculation was made, the treated portion of the stem was again rolled gently between the fingers and the thumb to facilitate the movement of the plant juices and the bacteria. More than 24 shoots were so inoculated. On all the raspberry shoots so treated, crown galls appeared only at the point of

inoculation. There was no visible evidence of crown-gall tissue in the form of strands of globular masses in any other part of the water-soaked area of the stem. The crown galls were large and often split the stem at the point of inoculation. The pith of the water-soaked region of the stem frequently showed signs of necrosis.

Figure 7 (Pl. XVII) shows a shoot of a raspberry plant, a part of which was water-soaked by gentle pressure and then inoculated in that region with *B. tumefaciens* by a single prick of a needle. The water-soaked portion of the shoot is indicated by two marks made with India ink. A crown gall appeared only at the point of inoculation. Free-hand sections of these stems above and below the crown gall were studied, but no indication of a crown-gall tissue appeared.

Figure 8 (Pl. XVII) shows the development of a crown gall in the water-soaked area of the stem. The new growth split the stem. A scar due to the injury induced by squeezing the shoot appears above the crown gall.

It appears from these experiments that the production of the so-called secondary tumors and strands is not facilitated by water soaking. The bacteria which migrate because of the gentle squeezing of the inoculated stem must require, it appears, an additional factor to bring about the proliferation of the cells in parts distant from the point of inoculation. Injury, accidentally induced in the water-soaked area due to excessive pressure, has not resulted in crown-gall formation other than at the point of inoculation.

THE EFFECT OF B. TUMEFACIENS INTRODUCED INTO SLITS MADE IN YOUNG STEMS

In these experiments I have used rapidly growing, and slowly growing shoots of the geranium and rose, and petioles of *Ricinus*. The apices of the branches were slit with a sterilized knife for a distance of from 10 to 12 cm. In many cases the slit involved the petioles of the youngest leaf. The depth of the cut was approximately to the center of the pith. *B. tumefaciens* was then introduced by means of a sterilized needle covered with bacteria and introduced between the cut surfaces of the stem. When a sufficient quantity of the bacteria was left in the slit, the stem was gently pressed until the bacteria were uniformly spread over the cut surfaces. Forty experiments of this kind were made. The plants were examined daily, and a large number of these were photographed at intervals of from 7 to 10 days. In the rapidly growing shoots it was noticed, shortly after the inoculations were made, that the cut surfaces began to separate; at the same time a proliferation of cells occurred in the cambium region along the surfaces. These growths increased in size and finally fused with each

other. After a period of from 5 to 6 weeks the cut surfaces began to lie in the same plane and were entirely covered with many small nodules which fused into a mass of crown-gall tissue.

Figures 9 and 10 (Pl. XVIII) show an early and a late view respectively of a growing stem of a geranium, slit for a distance of 12 cm. and extending into the petiole of a young leaf. These figures represent two of a series of photographs made at intervals of a week. At the end of a month a distinct hyperplasia appeared on the cut surfaces (Fig. 9, Pl. XVIII). At the end of seven weeks, the end of the experiment, the entire surface was covered with numerous intumescences which had fused into a uniform mass of crown-gall tissue on the cut surfaces of the stem and petiole. The petiole died five weeks after the inoculation was made, but remained attached to the stem. It was frequently noticed in these experiments that the terminal portion of the stem continued growing and a crown gall appeared at its apex (Fig. 10, Pl. XVIII). The significance of this is discussed below.

In slowly growing geranium stems the cut surfaces begin to separate slowly. Small intumescences appear at the nodes or at the axils of the leaves. These plants failed to develop crown-gall tissue on the entire inoculated surfaces. Figure 11 (Pl. XVIII) is a photograph of a geranium plant inoculated on February 19, 1924, and photographed for the last time on May 3 of the same year. Three months after inoculation the leaves withered. The crown galls remained isolated bodies at or near the leaf axils.

Young shoots of the rambler rose, slit and inoculated in a similar manner, form crown-gall tissue on the inoculated surfaces. Occasionally, however, globular masses of crown-gall tissue appear on the cut surfaces (Fig. 12, Pl. XVII). Young petioles of *Ricinus* likewise give similar results, although, as in the rose, globular crown galls frequently form on the cut surfaces (Fig. 13, Pl. XVII). These results suggested the possibility of inducing similar masses of crown-gall tissue internally by introducing *B. tumefaciens* into a long channel in the stem of a plant. The internal development of such globular masses of crown-gall tissues would bring about structures similar to those swellings described as secondary tumors in this disease.

LONG PERFORATIONS INOCULATED WITH B. TUMEFACIENS

The effects of introducing *B. tumefaciens* into long perforations made in the stems of various plants were studied. For this experiment twenty tobacco plants, twenty geranium plants, and twelve shoots of a rambler rose were used. A stiff wire, 40 cm. long, was pointed and by means of a rotary motion was thrust through the upper portions of the stem of the plant for a distance of from 10 to 20 cm. This needle was moved up and

down in the tissue a number of times to widen the channel thus formed. Without removing the needle from the stem, a culture of *B. tumefaciens* was smeared over the tip and upper part of the needle. The needle was again moved up and down in the channel. After the needle was withdrawn, *B. tumefaciens* was applied to the entire surface of the needle and it was again introduced throughout the length of the perforation. Introducing a suspension of the organism in water was not entirely satisfactory. The fluid ran down on one side of the puncture, a large number of the bacteria was lost, and only a limited portion of the internal surface of the channel was infected.

Two months after the inoculations were made all the plants showed crown galls at the points where the needle perforated the surfaces of the stem. The size of the crown gall appeared to be in proportion to the age of the tissue (6). Larger galls invariably appeared at the upper end of the stem.

Figure 14 (Pl. XVIII) is a photograph of a representative specimen of the tobacco stems used in my cultures. It shows the result of an inoculation made two months previously. At each point of inoculation, "A" and "B," distinct globular crown galls appear. Not infrequently leafy shoots covered these crown galls. No distinct globular mass of crown-gall tissue developed along the channel between the wounds made by the inoculating needle on the surface of the stem.

Longitudinal sections made through the line of inoculation on the stem show a distinct proliferation of cells which almost closes up the channel. Such a section of the stem made through, and extending from the points "A" to "B" in figure 14, is shown in figure 15 (Pl. XVIII). The new internal growth consists of a cylinder of tissue around the track made by the needle. In the center of the cylinder of this newly-formed tissue is a necrotic area. Differentiation of the new tissue occurs, resulting in the development of vascular elements. At the upper end of the section, at "A[1]", woody elements may be seen extending into the pith. In the region below "A[1]", a distinct cylindrical layer of tissue may be observed. The tissue surrounding this cylinder is apparently normal pith. Microscopic examination was made of pieces of pith through which the inoculating needle had passed. Figure 16 (Pl. XIX) represents a quadrant portion of a cross section of the pith through the region of inoculation. The irregularly-shaped wound filled with necrotic material is seen on the upper left in the figure, and is marked "C." The cross section shows the development of at least two distinct zones around the region of inoculation. Immediately surrounding the wound, small parenchymatous cells appear which have a fine granular cytoplasm and apparently normal

nuclei. Here the direction of growth appears to be radial. On the periphery of this zone another layer of cells appears. These are smaller, but form a band of tissue in cross section equal to the first zone. This layer of cells appears to be intercalated between the first zone and the apparently normal pith which follows. Figure 17 (Pl. XIX) shows an enlargement of the boundary portion between the first and second zones. The arched layers of cells are suggestive of pressure.

Control plants through which channels were bored from one to two months previously, but which were not inoculated, show changes slightly different. A cylinder of new cells is formed around the wound. The rate of growth appears to be much slower. No second or middle zone appears. The channel in the control plants remains large, and destroyed cells are visible in it.

A longitudinal section (Fig. 18, Pl. XIX) through the pith in this section of inoculation with *B. tumefaciens* shows more clearly the existence of a rudimentary but aberrant organization of fibrovascular bundles. A layer of cells, similar to that seen in figure 16 (Pl. XIX), is found around the opening made by the inoculating needle. The second or middle zone is not well marked in the longitudinal section; nevertheless, it shows vascular fibers three to four cells in thickness. These strands are made up of elongated cells which have smooth or reticulated walls. Two nuclei are visible in many cells. Figure 19 (Pl. XIX) shows these vessels and the associated parenchyma cells under higher magnification. The nuclei appear as two small bodies in the center of each cell. Figure 20 (Pl. XIX) shows the cells with reticulated walls.

Similar results were obtained in my studies on the geranium. Crown galls appeared at the points where the needle perforated the surface. No tumors of a leafy or globular variety appeared between the points where the needle perforated the surface of the stem. The microscopic studies of longitudinal and cross sections of the geranium stem give results similar to those obtained with the tobacco plant. The course of the inoculating needle is marked by a line of necrotic tissue. The cells here are not as markedly oriented as they appear to be in the tobacco stems similarly treated. Differentiation of the newly formed tissue occurs. A number of cells with reticulated markings are found in the middle zone.

Rose shoots inoculated through a channel made in the pith frequently form a globular crown gall at one end of the channel. The end of the channel occupied by the crown gall is generally the younger portion. The older portion of the stem forms a scar. The crown-gall tissue in the surrounding parts along the channel is similar to that described for the tobacco and geranium.

It appears from these sections of the tobacco, geranium, and rose stems that strands of crown-gall tissue can be produced by such inoculations as I have described above. When the inoculation is along a straight path, the new tissue resulting from the inoculation forms a strand-like body in which some of the elements of a conducting system appear. However, no globular growths of crown-gall tissue ever appeared in my cultures between the points of the injured surfaces. These results suggest that the bacteria, in their migration from the primary point of inoculation, require some other factor before they may sufficiently stimulate the normal cells to form globular masses of crown-gall tissue. One of these factors may be an increased supply of oxygen. Transverse punctures intersecting the longitudinal puncture should be made with a sterile needle to test the effect of an additional injury. I have observed that when two longitudinal intersecting punctures are made in the pith of geranium stem, the growth of the crown-gall tissue appears to be more active at the intersection. A relatively large mass of crown-gall tissue is formed in the stem at this point. Even in these cases no distinct globular mass of crown-gall tissue becomes visible on the surface.

INOCULATION OF YOUNG TISSUES

Crown galls that seem to arise at a point distant from the original seat of inoculation have been described. Smith (16) maintains that these galls are "secondary tumors" and borrows this term from the animal pathologist who uses the term to express the relationship existing between a malignant cancer and the growths which arise from it in other parts of the body by means of embolisms. Robinson and Walkden (15) and Riker (13, 14) hold that these so-called secondary growths in crown galls are due to the migration of bacteria which directly stimulate the cells distant from the points of inoculation.

For two years I have studied the effects of inoculating the first two internodal spaces below the bud of over 200 young *Ricinus* plants. These plants were immature at the time of inoculation, barely a foot above the ground. The bacteria were introduced by a single needle puncture. I have observed in these plants, in a limited number of cases, the development of crown galls on the uninoculated node.

Figure 21 (Pl. XVIII) is a photograph of a *Ricinus* stem that was inoculated in the internodal space below the bud. Shortly after the gall appeared, it was noted that the surface of the node above became slightly enlarged on the side inoculated and directly above the gall. There was no visible evidence of any continuity between these two. Two months later, October 21, 1922, when the photograph was made, a distinct smooth swelling ap-

peared on the node; and the two growths were visibly connected by a thickening which formed a ridge on the stem. A longitudinal section of the stem (Fig. 22, Pl. XVIII) shows a distinct hyperplasia of the wood and cortex between the gall and the node.

Figure 23 (Pl. XVIII) shows an older stem inoculated four months previously with *B. tumefaciens* in the two uppermost internodal spaces. Here a third crown gall on the node is shown between the two inoculated internodal spaces. The development was similar to that described above. This crown gall on the node appeared first like a little smooth swelling. As its development proceeded it burst through the epidermis and formed a crown gall not unlike the preceding galls that appear at the seats of inoculation. Longitudinal sections of this stem (Fig. 24, Pl. XVIII) through these galls show a thickening of the meristem with subsequent development of the wood, cortex, and epidermis. These thickenings of the tissue extend in all directions from the point of inoculation. The greatest growth appears to be parallel with the long axis of the stem and on the side where the inoculation was made.

It appears that after inoculation the growth of the crown-gall tissue proceeds in all directions. It is greatest in the vertical axis, as shown by a comparison of the thickness of the wood and cortex of the inoculated and uninoculated sides of the stem. When the longitudinal growth of the crown-gall tissue reaches the node, it ceases. It seems that the limit of its longitudinal growth is reached simultaneously with the attainment of the vertical maximum growth of the tissues of the internodal space. The new direction of growth seems to change at the node. This is at right angles to the axis of the stem, forming a globular mass of crown-gall tissue. The stimulated cambium grows faster than the stimulated cortical cells, and the crown gall bursts through the epidermis, which is slowest to respond, thus forming a gall with a rough surface. It appears that this is the fate of all the smooth swellings if their development is not interfered with. It may be argued that the bacteria, in their migration from the point of inoculation, find a more suitable type of cell for stimulation in the tissues of the node rather than at the internode. I have found no evidence that these so-called secondary galls are due to the migration of the bacteria from the point of inoculation. There appears to be no adequate explanation for the cessation of the movement of the migratory organism and stimulation of cells beyond the region of the node. I have not found these so-called secondary galls in nodes beyond the one above, bounding the inoculated internode. I have been able to isolate *B. tumefaciens* from the portion of the stem above the gall on a branch of rubber plant (5).

It appears that these so-called secondary galls are not the result of permeation of crown-gall tissue as I shall point out below. The entire

meristematic tissue of the inoculated side of the stem is involved. It appears that these hyperplasias are growths directly associated with the development and elongation of the stem and that when the main axis of growth occurs in the vertical direction, the elongation of the hyperplasia in the internode proceeds as rapidly as that of the rest of the stem. No visible evidence of these growths appears at this time. When the cells can no longer grow vertically, division and growth of the cells occur parallel with, and then at right angles to, the long axis of the stem; a smooth swelling occurs along the internode from the region of inoculation to the node. A swelling, with subsequent typical crown-gall formation, results at the node.

I inoculated the internodal spaces of the older portions of the stems of 60 *Ricinus* plants as controls. In no case have I found a crown gall in an uninoculated node. Fifty young tomato stems (var. Ponderosa), measuring a foot in height, were inoculated several inches above the ground. In no case have I found a growth of crown-gall tissue attributable to an inoculation at some distant point.

A more interesting case of these extension growths associated with inoculations with *B. tumefaciens* is seen in young, growing petioles of *Ricinus*. These petioles, as mentioned above, were less than 12 cm. long at the time of inoculation. Figure 25 (Pl. XVIII) represents one of a large number of petioles which was so inoculated. A globular crown gall appeared at "A," the point of inoculation, in about ten days. Shortly after that a series of protuberances appeared in succession. These were formed on the side of the inoculated petiole. Each protuberance first gives the appearance of a small swelling below the surface of the epidermis which appears to be stretched. It then cracks, and the rough crown-gall tissue appears under the surface. A similar condition occurs at the older portion of the petiole at "B," inoculated at the same time as "A." Two small protuberances are noted here, the large one extending visibly from the mass of crown-gall tissue at the point of inoculation.

Many of these petioles were fixed, and microtome sections were made shortly after these smooth swellings developed. Figures 26, 27, and 28 (Pl. XX) represent a series of photographs of the tissue between two such swellings as are shown at "A" in figure 25. No distinct permeating tumor strands are found between these small protuberances. The fibrovascular bundles, the cortex, and, to a slighter degree, the epidermal layer have all increased in thickness by increasing the number of elements in each layer. The tissue consists of elongated crown-gall cells. Figure 29 (Pl. XX) represents, under higher magnification, the pith side of the fibrovascular bundles of the section shown in figure 27. The cells are smaller in diameter but

longer. They are more numerous and represent a distinct hyperplasia. Nuclei are present in the cells. Cells with reticulated markings appear in abundance, although spiral vessels are few in number. The side of the petiole opposite to the point of inoculation is normal (Figs. 28 and 30, Pl. XX). The section of the fibrovascular bundles shows normal spiral and reticulated vessels. The cortex and epidermis are also normal.

A close examination of the protuberances or the sub-epidermal swellings (Figs. 26 and 31, Pl. XX) shows that they are made up of a number of parenchymatous cells and fibrovascular bundles. These are surrounded by cortical cells which are covered in turn by one or several layers of epidermal cells. The growth of the cortical and epidermal cells keeps pace for a time with that of the cambium elements. In many of these swellings the rate of growth of the isolated masses of cambium is greater than that of the cortical cells. The latter show signs of the effects of pressure in the region of contact. In figure 31 (Pl. XX) the upper crown gall illustrates this point. The lower crown gall in the figure shows the nodule of woody elements broken through the cortex and epidermis. That none of these nodules of differentiated tissues are strands is clearly shown by serial sections.

Young, growing petioles of *Ricinus*, when inoculated at two or more points ½ cm. apart, develop crown galls at the point of inoculation as described above; and, in addition, the tissue between the points of inoculation becomes visibly swollen. Microscopic examination of this tissue reveals the same type of growth as is shown in series figures 26 to 28 (Pl. XX). A cross section of a petiole between and near one of the points of inoculation is shown in figure 32 (Pl. XVIII). The swelling is due to an increase in the cortex and fibrovascular elements. The pith cells also show considerable proliferation. In the region of the pith and near the fibrovascular bundles, a number of isolated tracheids appear. These are shown in figure 33 (Pl. XVIII). These are not tumor strands but form a part of the new growth and result from a differentiation of the tissue.

Very young tissue inoculated with the crown-gall organism produces a hyperplasia which appears to grow not only at the point of inoculation, but also parallel with the direction of the developing organ. This must not be considered as an invasive growth. Only part of the crown-gall tissues forms a globular mass of cells. The rest grows parallel and keeps pace with the growth of the normal parts of the organ. In these petioles, as in the stems noted above, growth of the crown-gall tissue occurs simultaneously with the growth and elongation of the organ, and not subsequent to it. It appears from the study of these petioles that the structures that have been referred to as strands and secondary tumors are elongated growths of the crown-gall tissue. These extension-growths exceed the normal growth of

the organ, in which case protuberances or sub-epidermal swellings and ultimately crown galls appear at a point distant from the region of inoculation. These growths are parts of the crown gall at the initial point of inoculation and develop simultaneously with it. In this they differ from the permeation metastases in animal and human cancer. These swellings or protuberances of crown galls at points distant from the region of inoculation do not form after inoculation of older petioles and stems.

BUD INOCULATIONS

I have also inoculated terminal buds of *Ricinus* for the purpose of studying the crown galls on the leaves after the buds develop. In a large majority of cases the inoculation results in the formation of a globular crown gall at the bud region with subsequent dwarfing of the plant (Fig. 34, Pl. XVIII). In a few cases of inoculated buds I have been able to secure overgrowths on the petioles of the leaves of *Ricinus* plants when the buds had developed. Figure 35 (Pl. XVIII) shows the apical portion of a *Ricinus* plant. The terminal bud was inoculated July 28, 1924, the photograph made October 13, 1924. The bud had opened by this time, but only one petiole was infected with crown gall. The new terminal bud and node were transformed and axilliary buds began to grow (see 9, 10). These figures confirm the observations of Robinson and Walkden (15) and Riker (13) for the Paris daisy and the tomato respectively.

I have made a large number of cross sections of the petiole shown in figure 35 (Pl. XVIII) between the crown gall at the blade and the base of the petiole. There is no indication of any strands of crown-gall tissue between the gall at the base of the petiole and the crown gall at the blade end.

Figure 36 (Pl. XX) represents one of a large number of sections made of the petiole shown at "A" in figure 35 (Pl. XVIII). In all these sections the cortex and fibrovascular bundles were examined carefully for the presence of crown-gall tissue. Sections in the crown-gall region made at "B" (Fig. 35, Pl. XVIII) are shown in figure 37 (Pl. XX). A distinct increase in the number of fibrovascular elements and an aberrant arrangement of these parts are shown. There is no continuity of crown-gall tissue between the crown gall on the stems and the growth of the petiole at "B."

SUMMARY

1. The so-called secondary tumors and strands in the crown-gall disease are formed only in very young stems and petioles.

2. The so-called secondary tumors and strands result from the growth and elongation of the immature tissues. The young infected tissues keep

pace for a time with the elongation and development of the inoculated organ.

3. The so-called secondary tumors and strands begin to form simultaneously with the globular crown galls at the seat of the inoculation and depend upon the development of the organ for their elongation. Permeation metastases in human cancer occur through lymph channels of mature structures (2).

CANCER RESEARCH LABORATORY,
MONTEFIORE HOSPITAL, NEW YORK.

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DESCRIPTION OF PLATES

The photomicrographs were made with the aid of the Bausch & Lomb D. L. type camera. Zeiss objectives and compensating oculars were used as follows: For figure 20—obj. 8, oc. 8, bellows 20 cm.; for figures 17, 19, 29, 30, 33—obj. 16, oc. 8; for figures 16, 18, 26, 27, 28, 31, 32, 36, 37—obj. A_2, oc. 1, bellows 26 cm.

PLATE XVII

FIGURES 1–2. Shoot of rambler rose inoculated with *B. tumefaciens* in back of the region of elongation at two or more points ½ cm. apart, showing a confluence of galls.

FIGURE 3. Longitudinal section of galls shown in figure 2.

FIGURE 4. Crown galls on a young shoot of rambler rose resulting from two inoculations made with *B. tumefaciens* 3 cm. apart.

FIGURES 5–6. Ventral and dorsal view of a leaf of the rubber plant inoculated on May 27, 1922, at a number of points ½ cm. apart. Photographs made on November 1, 1924, show no swellings between the points of inoculation.

FIGURES 7–8. Crown galls on shoots of raspberry (var. Cuthbert) inoculated with *B. tumefaciens* in water-soaked area. Stems between arrows were water-soaked by gentle pressure.

FIGURE 12. Globular crown galls in the inoculated slit surfaces of a shoot of the rambler rose.

FIGURE 13. Globular crown galls on the inoculated slit surfaces of a petiole of *Ricinus*.

PLATE XVIII

FIGURE 9. A photograph showing an early effect of inoculating *B. tumefaciens* into a slit made in the growing portion of a geranium plant.

FIGURE 10. Same plant as shown in figure 9 a month later.

FIGURE 11. Slow-growing geranium with slit apical region inoculated with *B. tumefaciens*, showing crown galls at leaf axils 75 days after inoculation.

FIGURE 14. Tobacco stem perforated and inoculated with *B. tumefaciens*. The distance between "A" and "B" shows the length of the perforation.

FIGURE 15. Longitudinal section of a tobacco stem showing the internal changes in the pith resulting from the perforation and the inoculation with *B. tumefaciens*.

FIGURE 21. *Ricinus* stem inoculated in the internodal space below the terminal bud showing swelling at the node a month later.

FIGURE 22. Longitudinal section through the stem; the swelling is indicated by the arrow.

FIGURE 23. A crown gall on the node resulting from inoculation with *B. tumefaciens* into the young internodal spaces below and above it. The photograph was taken approximately four months after inoculation.

FIGURE 24. Longitudinal section of the stem shown in figure 23.

FIGURE 25. Crown galls on the petiole of *Ricinus* inoculated with *B. tumefaciens* at the points "A" and "B" with small protuberances developing from them.

FIGURE 34. *Ricinus* plant inoculated with *B. tumefaciens* in the terminal bud.

FIGURE 35. Crown gall on the distal end of the petiole resulting from an inoculation of the terminal bud with *B. tumefaciens*.

PLATE XIX

FIGURE 16. Microscopic view of a cross section through the pith of a tobacco stem shown in figure 14 (Pl. XVIII); "C" indicates region of inoculation.

FIGURE 17. Same as figure 16, showing part between first and second zone under higher magnification (see text).

FIGURE 18. Longitudinal section of the pith shown in figure 14 (Pl. XVIII).

FIGURE 19. Longitudinal section showing differentiation of the middle zone (see text).

FIGURE 20. Longitudinal section showing the reticulate cells under higher magnification. (Same as fig. 19.)

FIGURE 32. Microscopic view of cross section of a very young *Ricinus* petiole between two points of inoculation.

FIGURE 33. Same as figure 32,, showing cross section of tracheids in pith under higher magnification.

PLATE XX

FIGURES 26, 27 & 28. A series of sections between two protuberances shown in figure 25 (Pl. XVIII) at "A."

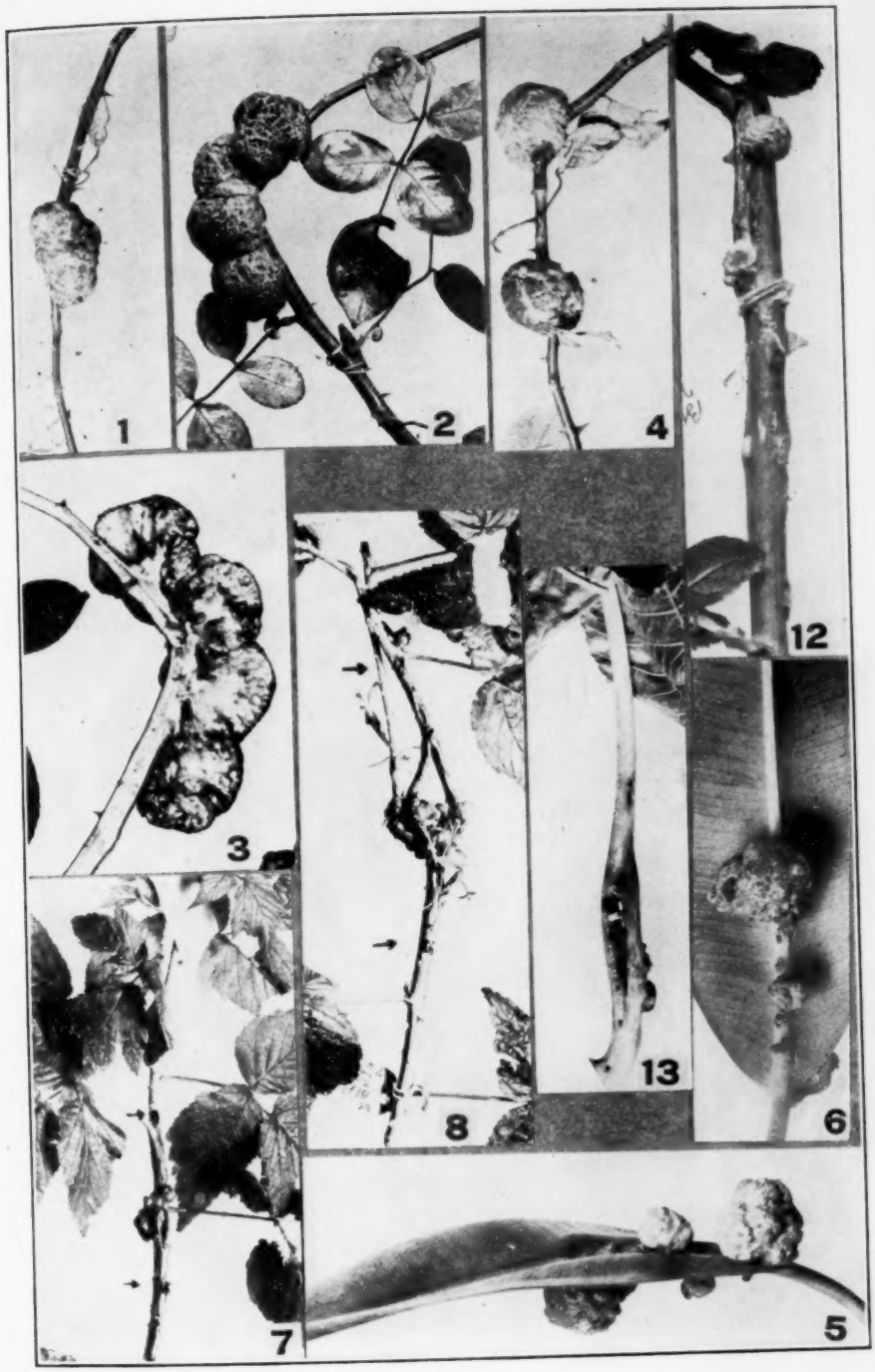
FIGURE 29. A part of the fibrovascular bundles lying near the pith on the crown-gall side of the petiole shown in figure 27.

FIGURE 30. The normal fibrovascular bundles of the petiole on the side opposite to the crown gall shown in figure 27.

FIGURE 31. Longitudinal section of a crown gall on a *Ricinus* petiole in which the woody nodule has broken through the thickened cortex.

FIGURE 36. Cross section of a petiole shown in figure 35 (Pl. XVIII) with normal arrangement of the fibrovascular bundles.

FIGURE 37. Cross section of the same petiole near the crown gall.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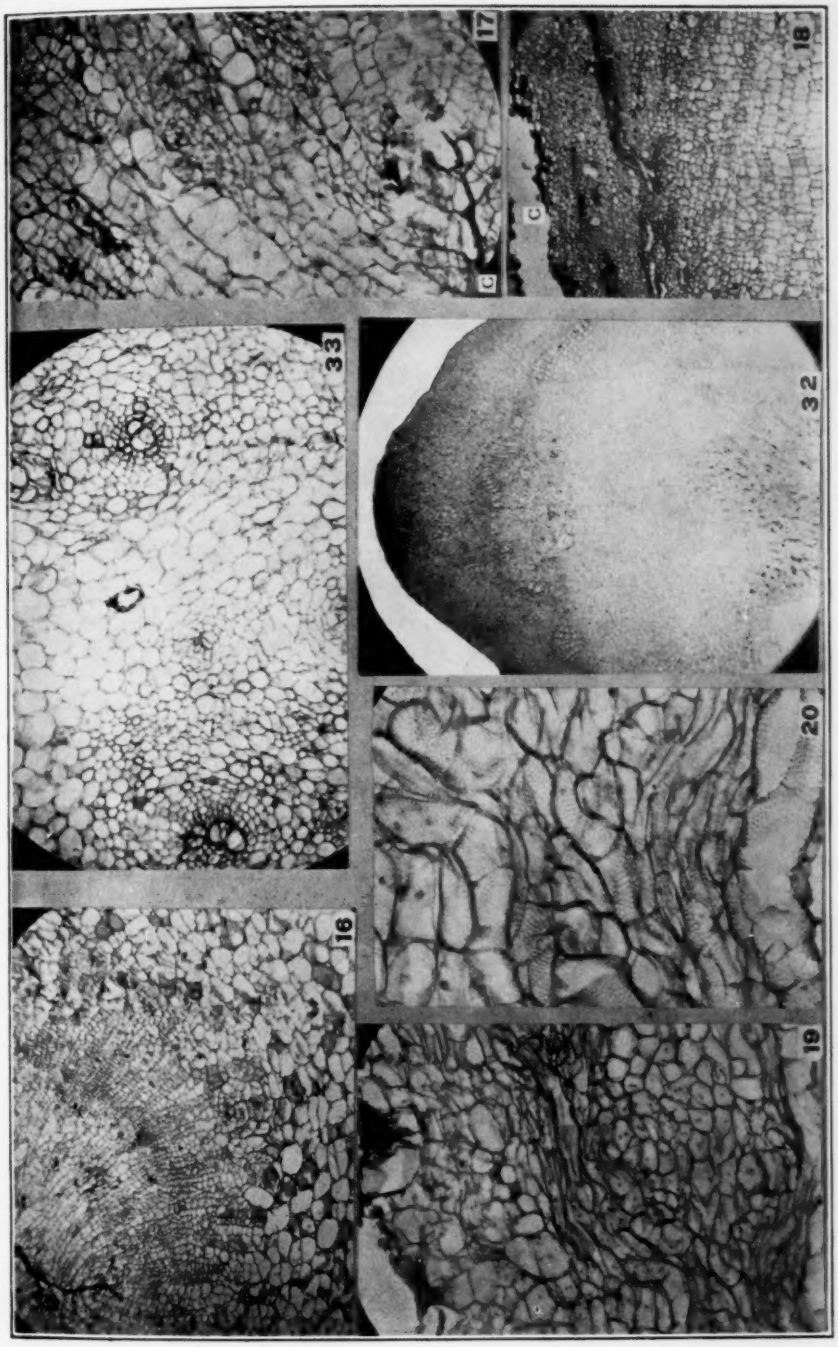
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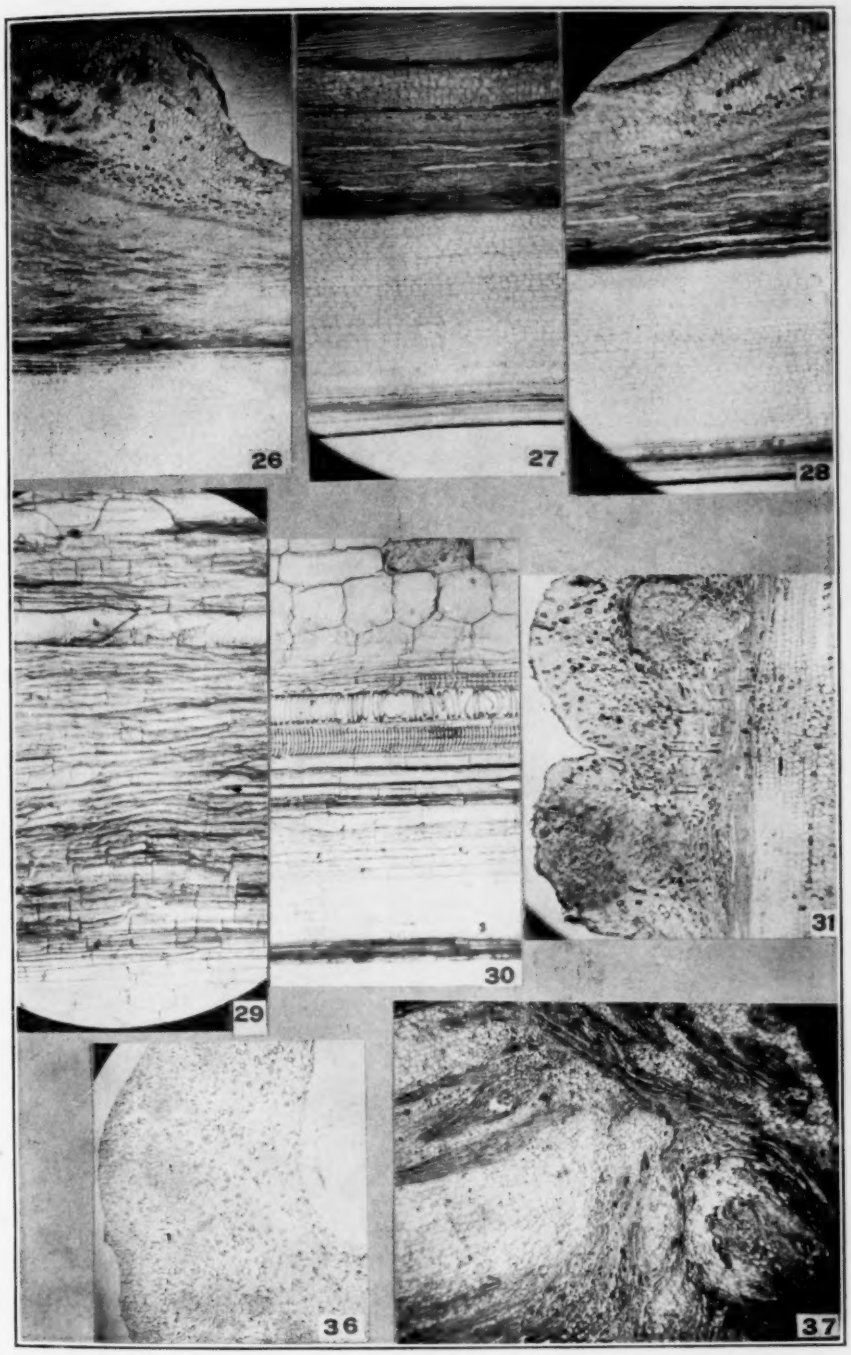
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CLADOSPORIUM SPOT OF COWPEA[1]

MAX W. GARDNER[2]

WITH PLATES XXI TO XXIII

In variety plots of cowpeas (*Vigna sinensis* Endl.) grown at Lafayette, Indiana, a peculiar spotting of the pods of the Early Buff variety was observed in August, 1923, and again in August, 1924. This spotting was in the nature of irregular, black, scabby lesions, distinctly different from the smooth or sunken, circular, maroon lesions of the bacterial spot disease[3] which was being investigated at that time. The trouble was confined to the Early Buff variety, and a more careful inspection further revealed that the peduncles and to a less degree the growing tips of the stems of this variety were thickly beset with small, sunken, purplish lesions which were likewise not to be found on any other variety in the plots. Microscopic, cultural, and inoculation tests proved that this disease was due to an apparently undescribed species of *Cladosporium*, herein designated as *Cladosporium vignae* n. sp., which evinces a predilection for very young, rapidly growing tissues and which has been found to be a seed-borne parasite.

SYMPTOMS

The lesions on the pods were conspicuous, dark purple or black, roughened or scabby spots, very irregular in shape and usually from 2 to 6 mm. in diameter, sometimes larger (Pl. XXI, figs. A to D). The margin of the lesion was irregularly lobed, slightly elevated, and dark purple in color, while the center of the lesion was slightly sunken, brown, tan, or grayish in color (Pl. XXI, fig. D) and sometimes covered with a velvety greenish layer, the spores and sporophores of the causal fungus. In older lesions the central tissue was corky and cracked (Pl. XXII, fig. F) and tended to scale or slough off. Late infection was in the form of numerous very small, black specks (Pl. XXI, fig. A). When the pods matured and became light colored, the lesions were much more conspicuous. This disease differs from bacterial spot in that the pod lesions were often scabby and were darker colored, more conspicuous, and, as a rule, more numerous.

[1] Contribution from the Botanical Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

[2] The writer is indebted to Prof. H. S. Jackson for advice and to Mr. James B. Kendrick for assistance. Dr. W. J. Morse, of the U. S. Bureau of Plant Industry, very kindly supplied seed of cowpea varieties.

[3] Gardner, Max W. and James B. Kendrick. Bacterial spot of cowpea and lima bean. Jour. Agr. Res. (In press.)

Owing to the fact that infection can occur, apparently, only when the pod is very young, the lesions usually interfered to some extent with the normal growth of the pod and hence caused considerable constriction and bending of the pods, the concavities being occupied by lesions (Pl. XXI, fig. A; Pl. XXII, figs. C and E). Heavy infection of very young pods sometimes resulted in their death. One case of a shrivelled dead ovary, 5 cm. in length, bearing dense, olive green, velvety masses of the spores and sporophores of the fungus was observed.

A large number of infected pods were opened and in a few instances the lesions were found penetrating through the pod wall into the seed. In one of these cases the fungus was found sporulating at the center of a reddish brown spot on the seed coat. In another case in which the lesion was on the ventral suture of the pod, to which the seeds are attached, the underlying seed was much stunted, and the fungus was found sporulating on the membranous inner lining of the pod. Other fungi, such as *Alternaria*, were often present in the old pod lesions.

In the field in the late summer and fall, infection of the peduncles was particularly abundant and conspicuous, and consisted of numerous elliptical, sharply and deeply sunken, dark purplish spots, thickly clustered toward the apical end of the peduncle (Pl. XXII, figs. A and B). These spots varied from 1 to 6 mm. in length, the larger ones being more distant from the apex, more scattered, and usually showing a tan center on which the greenish sporulation of the fungus was frequently visible. Older lesions were sometimes reddish brown or tan and were usually drawn out at either end into rather sharp points (lens-shaped). Occasionally peduncles were killed outright by the coalescence of the lesions.

Infection near the growing tip of the stem very much resembled the peduncle infection except that the lesions were smaller, less deeply sunken, and less numerous (Pl. XXII, fig. D). The lesions were dark purple at first and later developed a tan center, and were usually 1–3 mm. in length. Similar small, dark purple lesions occurred rather abundantly on the petioles, petiolules, and main veins of the leaves (Pl. XXII, fig. D). The lesions were often on the veins on the lower side of the leaf and by inhibiting growth were very likely to cause considerable distortion of the leaf. On the leaf blade very small, circular, sunken, dark purple- or maroon-bordered and tan-centered spots, 0.5 to 1 mm. in diameter, were formed (Pl. XXIII, fig. A). These were usually surrounded by a yellowish translucent margin. Similar lesions were produced on the stipules, bracts, and calyx. Under field conditions it may be said that in general the *Cladosporium* lesions were smaller, darker, and more sharply defined than bacterial spot lesions.

The first leaves of inoculated seedlings in the greenhouse were considerably distorted and curled upward by the numerous lesions. The latter were from 1 to 3 mm. in diameter, faded green or grayish, and were soon covered on both sides with the dusty spores of the fungus. Such leaves were soon killed and seedlings were sometimes killed outright by heavy infection. In cases of less destructive infection the leaf lesions were maroon- or purple-bordered, brown or tan spots, irregular in outline, sunken at the center, and about 2 mm. in diameter (Pl. XXIII, fig. D). Much crinkling and tearing of the leaves was produced (Pl. XXIII, fig. C).

Sharply sunken, lens-shaped, grayish or yellowish lesions, 2 to 4 mm. in length, developed in abundance on the hypocotyls and epicotyls of inoculated seedlings (Pl. XXIII, figs E, F, and G). At the center of the lesion the olive-green dusty layer of spores was produced. The lesions near the tip of the epicotyl became much larger, and in the case of seed-borne infection hypocotyl lesions up to 20 mm. in length were produced, usually emanating from the cotyledon scar and often penetrating half-way through the hypocotyl. Longitudinal fissures often accompanied these lesions. In older plants the lesions on the peduncles 5 days after inoculation were sharply sunken, elliptical, water-soaked spots with a faint brownish margin and about 1 mm. in length.

CAUSAL ORGANISM

Reference has been made to the presence of a *Cladosporium* sporulating at the centers of certain lesions. Poured plate isolations from the crushed tissue of pod lesions proved that these were not caused by *Bact. vignae*. Poured plate isolations from the fungous growth on the surface of pod, peduncle and stem lesions yielded similar *Cladosporium* colonies, as did also plates poured from the fungus on the small blighted pod 5 cm. in length, previously mentioned. Tissue plantings yielded the same fungus.

Portions of host tissue bearing lesions were cut out, immersed in alcohol and then in a 1 to 1,000 solution of mercuric chloride 1–2 minutes, rinsed in sterile water, and planted in poured plates of potato agar. What appeared to be the same *Cladosporium* developed on 6 of the 15 pod lesions, 9 of the 31 leaf-blade lesions, 3 of the 7 petiole lesions, 3 of the 18 stem lesions, and 12 of the 20 peduncle lesions thus tested. Usually the fungus sporulated directly on the upper surface of the tissue fragment. The same fungus was similarly isolated from a seed from an infected pod. By successful atomizer inoculation of seedlings with a spore suspension, the pathogenicity of the fungus was proved for cultures isolated by the poured-plate method from pod, peduncle, and stem lesions.

The colonies in plates of potato dextrose agar poured from a spore suspension were from 5 to 8 mm. in diameter in 3 days at room temperature,

and the surface of each colony was almost completely covered with spores, a circumstance which illustrates how promptly sporulation occurs. The mycelium remains prostrate and becomes olivaceous black, a fact to which no doubt the dark color of the host lesions is due (Pl. XXI, fig. E). The dusty sporulating surface approximates first the vetiver green, then the Lincoln green, and finally the deep grayish green of Ridgway's Color Standards. Excellent growth occurs on other media such as oat and lima bean agar.

Spore germination occurred in exposed water drops at room temperature, and the germ tubes were 1 to 4 times the spore length in 18 hours. In similar drops to which fragments of a cowpea leaflet were added, a higher percentage of the spores germinated, and the tubes were from 4 to 8 times the spore length in 18 hours. However, success was not always obtained in germination tests. Vigorous germination occurred in infection drops on the leaves of inoculated seedlings. In one trial in which vigorous germination occurred in a drop of tap water on a slide, many of the spores which had remained floating had at the end of 48 hours sent long germ tubes down into the liquid and branched chains of small spores up into the air. This illustrates again how promptly the fungus proceeds to spore production.

MORPHOLOGY AND TAXONOMY

The spores are produced in a cluster of branched chains at the end of an unbranched sporophore. In agar media there is no aggregation of hyphae preliminary to the production of sporophores, but the isolated hyphae emerge directly from the medium and produce an apical cluster of branched spore chains. Ten or more ultimate chains may be formed, each consisting usually of from 2 to 5 spores which are much smaller than the basal spores at the center of the cluster. The spores are light brown in color and are produced acrogenously, that is, the apical spore of each chain is the youngest. The spores produced on seedlings 5 days after inoculation varied in length from 7 to 22 μ while the width varied from 3 to 5 μ. In agar culture spores as short as 6 μ are formed. The spores are somewhat pointed at the ends and the smaller spores are lemon-shaped while the larger ones are cigar-shaped. On each end of the larger spores is a small, thickened disk, apparently the point of attachment to the sporophore or adjacent spores. The spores are usually one-celled, although the larger spores are sometimes two-celled. The spores are easily detached and carried by air currents as was indicated by the heavy seeding of new colonies obtained by blowing the breath across a plate colony. In germination the spores become swollen and may become darker brown and septate.

The sporophores from seedling lesions 5 days after inoculation varied from 40 to 80 μ in length and were darker brown and thicker walled than the spores. The sporophores are unbranched and contain one or two cross-walls and frequently a bend or crook where the sporophore has apparently continued its growth after producing a spore or spore chain. Spore production may begin before the sporophores are full grown. Cross sections of the lesions show that the sporophores are numerous and may be solitary or two or three in a group.

No record of any *Cladosporium* parasitic on cowpeas has been found, and the fungus under consideration differs from the few species described on legumes. The spores of *Cladosporium pisi* Cug. and Macch.[4] on *Pisum* are recorded as smaller with the smaller ones usually 2-celled. Attempts to infect field pea seedlings with the cowpea fungus have been unsuccessful. The spores of *Cladosporium infuscans* Thum.[5] on *Desmodium* are described as usually septate. *Cladosporium album* Dowson[6] described on *Lathyrus* differs in that it is white. Apparently the cowpea parasite is an undescribed species and for it the binomial, *Cladosporium vignae* n. sp., is suggested. It is described as follows:

Cladosporium vignae n. sp.

Sporophores solitary or in groups of two or three, unbranched, often with a bend or crook, septate, brown, 40 to 80 μ in length. Spores borne in an apical cluster of branched chains, acrogenous, light brown, ovoid to ellipsoidal, somewhat pointed at the ends, 7 to 22 μ by 3 to 5 μ, usually 1-celled, the larger spores sometimes 2-celled. In culture the submerged mycelium is olivaceous black, the dusty spore layer, vetiver, Lincoln, or deep grayish green. Parasitic on cowpea (*Vigna sinensis* Endl.), especially the Early Buff variety, producing black scabby lesions on the pods, purplish sunken lesions on the peduncles, stems, and petioles, and very small dark spots on the leaves. Also parasitic on *Vigna sesquipedalis* Wight. Only young growing tissues are susceptible. The fungus is seed-borne and seedling infection occurs. Type locality: LaFayette, Ind. Specimens deposited in the Pathological Collections, U. S. Bureau of Plant Industry, Washington, D. C.

[4] Cugini, G., and L. Macchiati. Notizie intorno agli insetti, acari & parassiti vegetali osservati nelle piante coltivate & spontanee del Modenese nell' anno 1890. Boll. d. R. Staz. Agraria di Modena **10**: 89–107. 1891.

[5] Saccardo, P. A. Sylloge fungorum **4**: 361. 1886.

[6] Dowson, W. J. Contributions from the Wisley laboratory. XLIV. A new disease of sweet peas. Jour. Roy. Hort. Soc. **49**: 211–221. 1924.

PATHOGENICITY

Atomizer inoculation of Early Buff cowpea seedlings in the greenhouse with a suspension of the spores resulted in abundant infection of the young rapidly growing organs. Nine seedlings one week old which were inoculated and placed in a moist chamber on October 11 showed numerous lesions on the young first leaves, epicotyls, and hypocotyls within 48 hours. These lesions increased in size to such an extent that at the end of one week 6 of the seedlings were dying. Abundant sporulation occurred on the lesions. No infection occurred on 5 seedlings sprayed with water and incubated as controls. The fungus was reisolated by plates poured from a suspension of the spores produced on an epicotyl lesion, and with this culture infection was obtained in later inoculations.

Ten New Era and 6 Whippoorwill cowpea seedlings one week old were similarly inoculated on October 11. Numerous very small, black, sunken, shiny spots, 0.5 mm. in diameter, appeared on the first leaves of both varieties (Pl. XXIII, fig. B) and a few small elliptical lesions from 0.5 to 1 mm. in length developed on the epicotyls and hypocotyls just above and below the cotyledon scars. Necrosis and blackening occurred much more promptly in the lesions on these varieties than on the Early Buff, but the lesions remained much smaller and showed little or no sporulation of the fungus. New Era and Whippoorwill seedlings sprayed with water as controls remained free from infection. These tests showed that these two varieties were not immune but were not nearly as susceptible as the Early Buff variety.

To illustrate how soon the tissues become resistant to this fungus, inoculation tests made one week later with seedlings of the three varieties planted at the same time as those used in the tests described above, hence 2 weeks old, resulted in no infection. No new leaves had developed on these seedlings and the first leaves, epicotyls, and hypocotyls had evidently become somewhat resistant. Nine and ten days later, inoculations on similar seedlings, now over three weeks old, and some with the first compound leaves unfolding, resulted in abundant infection of the unfolding leaves of certain of the Early Buff and New Era seedlings (Pl. XXIII, fig. C). Petioles, stipules, and bracts were also infected. With one or two exceptions no infection occurred on the stems or first leaves except on the ventral or upper surface of the very short petiole of the first leaf, a region which apparently remains susceptible. Lesions on this area were purplish and lace-like, and from two of these the fungus was cultured. In one case a few lesions developed on the stem near the tip and in another case on the stipule of an older leaf.

In later inoculations of seedlings of which the second compound leaf was unfolding, infection occurred only on the latter and not on the older

leaves, so that one is led to conclude that, in general, only the young growing organs are susceptible, a condition which field observation likewise suggests. Under favorable conditions, however, infection of such organs occurs with great rapidity and virulence, and visible lesions already causing crinkling of the leaves may be present within 48 hours after inoculation. Inoculation of older plants resulted in numerous lesions on the very young leaflets, stem tips, peduncles, and bracts, and in 5 days the fungus was sporulating profusely on the bracts surrounding the immature floral organs.

In order to determine varietal susceptibility and host range, greenhouse inoculation tests were made upon seedlings of 15 varieties of cowpeas and two related species with the result that the Early Buff variety proved most susceptible while the Progressive White variety ranked rather close to the former in its susceptibility. Next to these were the Columbia variety and the related species, asparagus bean (*Vigna sesquipedalis* Wight). Then followed a group of less susceptible varieties consisting of Groit, Whippoorwill, Large Blackeye, Early Red, Victor, New Era, Red Ripper, Clay, Iron, and Brabham. The Taylor and Early Black varieties and the species catjang (*Vigna catjang* Walp.) showed a high degree of resistance and the Arlington variety was almost immune. On these resistant forms, very minute lesions, in fact scarcely visible specks or dots, were noted the fourth day but were more or less healed over and almost invisible by the eleventh day after inoculation.

No hosts other than the *Vigna* species have been found susceptible. The similarity of the disease to cucumber scab in its preference for young tissues suggested a relationship, but two attempts to infect cucumber seedlings were a total failure while with the same inoculum abundant infection of cowpea seedlings was obtained. Failure likewise attended similar inoculation tests with field pea, hyacinth bean, and four varieties of garden bean.

Field observation would indicate that the disease needs to be feared principally on the Early Buff variety of cowpeas. In 1924 the following varieties were arranged in small parallel plots as follows: Whippoorwill, Blackeye, Iron, Early Buff, catjang (*Vigna catjang*), Groit, New Era, and Early Red. In August when the disease was very conspicuous on the Early Buff, the vines from the adjacent rows of the Iron cowpeas and catjang peas were intermingled with the Early Buff plants and yet no lesions could be found on these nor on any of the other varieties.

The relation of the fungus to the host tissue has not been carefully studied. Microscopic examination of inoculated leaflets cleared in chloral hydrate and stained in safranin or Delafield's haematoxylin indicates that germ tubes from the spores do not enter the stomata. Sections through hypocotyl and pod lesions show that there is a collapse of the host cells in

the invaded region attended by an accumulation of the dark mycelium and a prompt sporulation of the parasite on the surface. Pod lesions are often accompanied by a limited hyperplasia of the underlying parenchyma cells. Hypocotyl and epicotyl lesions frequently involve the entire thickness of the cortex and occasionally cause a longitudinal fissure extending well into the pith.

SEED TRANSMISSION

Because of the very abundant pod infection (Pl. XXI, figs. A to D), the fungus may readily gain access to the seed both by direct mycelial invasion and by surface contamination with spores, sporophores, and mycelium during threshing. A case was previously described in which the fungus had invaded a seed under a pod lesion and was sporulating on the seed. Since, in its mode of germination, the cowpea seedling usually carries the seed coat up into the air on the cotyledons and frequently carries it higher still on the first leaves (Pl. XXIII, fig. E), any organism on or in the testa may readily gain access to the growing portions of the seedling.

In order to test the possibility of seed transmission of this fungus, seeds collected from under the lesions in diseased pods harvested one month previously were sown in pots of sterilized soil in the greenhouse. When these seedlings were 13 days old and the majority were still bearing their seed coats aloft, they were kept moist four days in a humid compartment in order to afford the fungus favorable conditions for infection. Within one week 11 of the 51 seedlings, or 21 per cent, were showing infection in the shape of elongated, sunken, hypocotyl lesions, from 5 to 15 mm. in length, some with the fungus sporulating at the center. In three cases the lesion extended downward from a cotyledon scar (Pl. XXIII, fig. F) and in two of these cases the cotyledon attached at this scar was infected and at its base bore the fungus in a sporulating condition (Pl. XXIII, fig. F). Lesions which occurred at the ground line or midway of the hypocotyl (Pl. XXIII, figs. E and G) had in some cases penetrated so deeply as to cause the seedling to bend over. In fact a number of the seedlings were soon killed by these primary lesions.

The causal fungus was isolated from five of these seedlings by pouring plates from the spores produced on the hypocotyl lesions and also in one instance from the spores produced on the base of an infected cotyledon. One of these cultures from a hypocotyl lesion was used in successful inoculation tests. These tests prove that the fungus is carried with the seed. The selection of seed from apparently healthy pods did not entirely eliminate the disease, however, as 4 out of 131 seedlings grown from such seed developed hypocotyl lesions. This is attributed to surface contamination of the seed.

Among seed lots of a number of varieties planted in pots in March, a few cases of what was in all probability seed-borne infection occurred in the Large Blackeye variety. The viability of the fungus at this date would indicate that it may very readily persist from season to season in the seed.

SUMMARY

The Cladosporium spot disease of cowpeas is characterized by blackened scabby spots on the pods, sunken purplish spots on the peduncles and stems, and small blackened spots on the leaves and bracts. The causative fungus has been isolated and its pathogenicity proved. It has been designated as *Cladosporium vignae* n. sp. The Early Buff is the most susceptible variety and is the only one on which the disease has been observed in the field. However, greenhouse inoculation tests were successful on 14 other varieties, of which the Progressive White proved to be very susceptible and the varieties, Early Black and Taylor and especially the Arlington, showed a high degree of resistance. *Vigna sesquipedalis* proved susceptible, while *Vigna catjang* showed high resistance. Only young growing tissues are susceptible to infection. The disease is seed-transmitted.

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LAFAYETTE, INDIANA.

DESCRIPTION OF PLATES

PLATE XXI

FIGS. A to D. Types of pod infection, Cladosporium spot of Early Buff cowpea. A and B are heavily infected pods somewhat distorted in their development by the presence of the lesions. Near the lower end of figure A, very small lesions may be seen. D shows large lesions with dark borders and tan centers.

FIG. E. Colonies of *Cladosporium vignae* 10 days old. Poured plate culture from spore suspension, potato dextrose agar. Enlarged × 2.

PLATE XXII

FIG. A. Three young peduncles showing the sharply sunken, purplish lesions. Enlarged × 2.

FIG. B. Lesions on older peduncles.

FIG. C. Lesions on a very young pod causing crooks and inhibiting the normal enlargement.

FIG. D. Lesions near the growing point of a stem and on petiole and leaflets.

FIG. E. Lesions on a young pod under which inhibition of seed development has resulted.

FIG. F. Conspicuous dry scabby lesions on a nearly mature pod. Enlarged × 2.

PLATE XXIII

FIG. A. Small, inconspicuous lesions on young leaf, as a result of natural field infection. Enlarged × 1½.

FIG. B. Very small, blackened lesions on the first leaf of a seedling of the less susceptible New Era variety as a result of atomizer inoculation made 6 days previously. These lesions did not increase further in size.

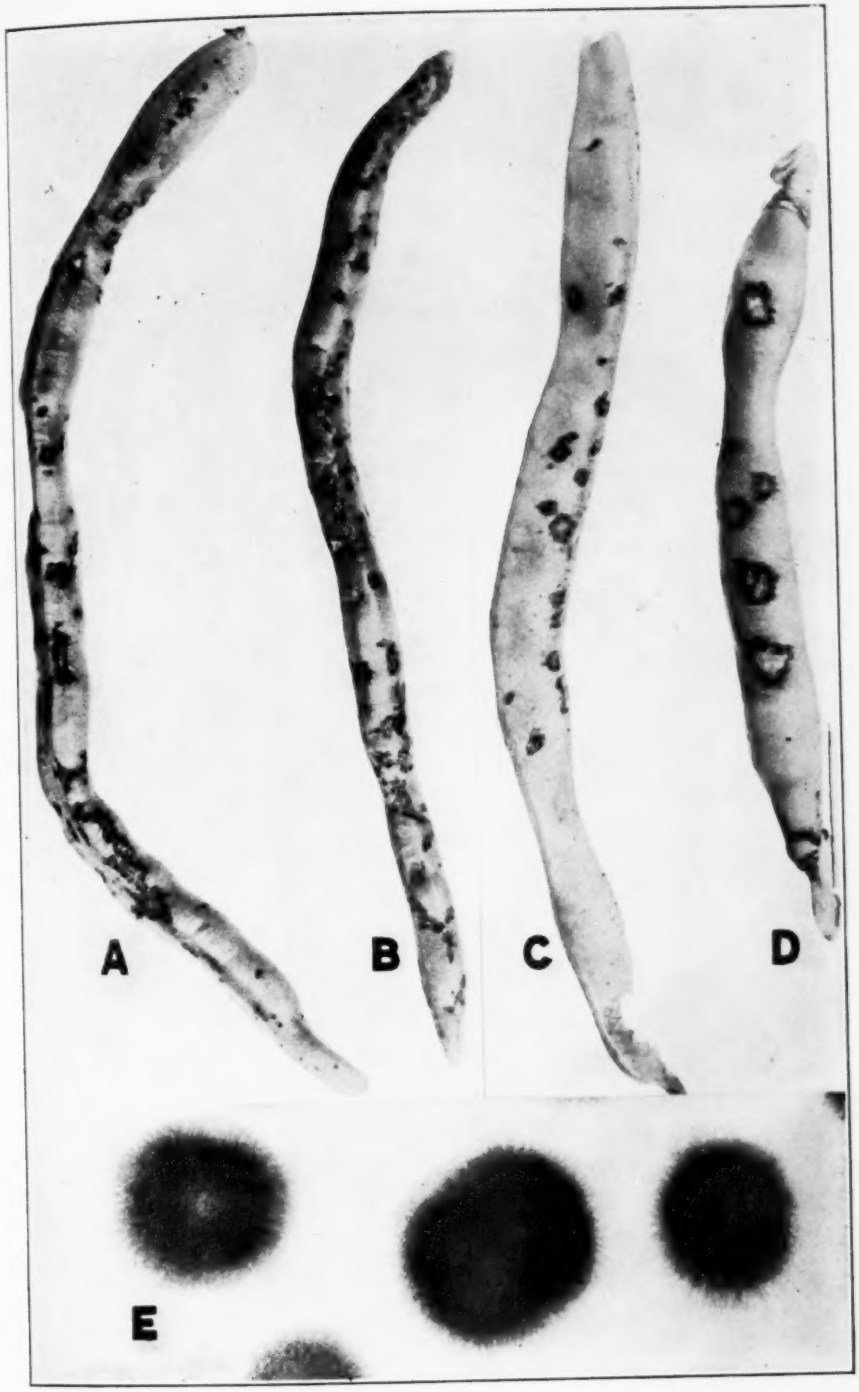
FIG. C. First compound leaf of Early Buff seedling showing large lesions resulting from atomizer inoculation made 16 days previously when the leaf was unfolding. Enlarged × 2.

FIG. D. Lesions on first leaf of Early Buff seedling inoculated after the leaf was full grown. Enlarged × 2.

FIG. E. Seedling showing primary lesion on hypocotyl as a result of seed-borne infection. The lesion penetrated so deeply as to cause the hypocotyl to break at that point. The seed coat has been carried up on the first leaves. Enlarged × 1½.

FIG. F. Seedling showing lesion at the cotyledon scar. The cotyledon to the left, which was attached to this scar, is infected at its basal end and the fungus is sporulating thereon. Enlarged × 1½.

FIG. G. Primary lesion on hypocotyl. Enlarged × 2.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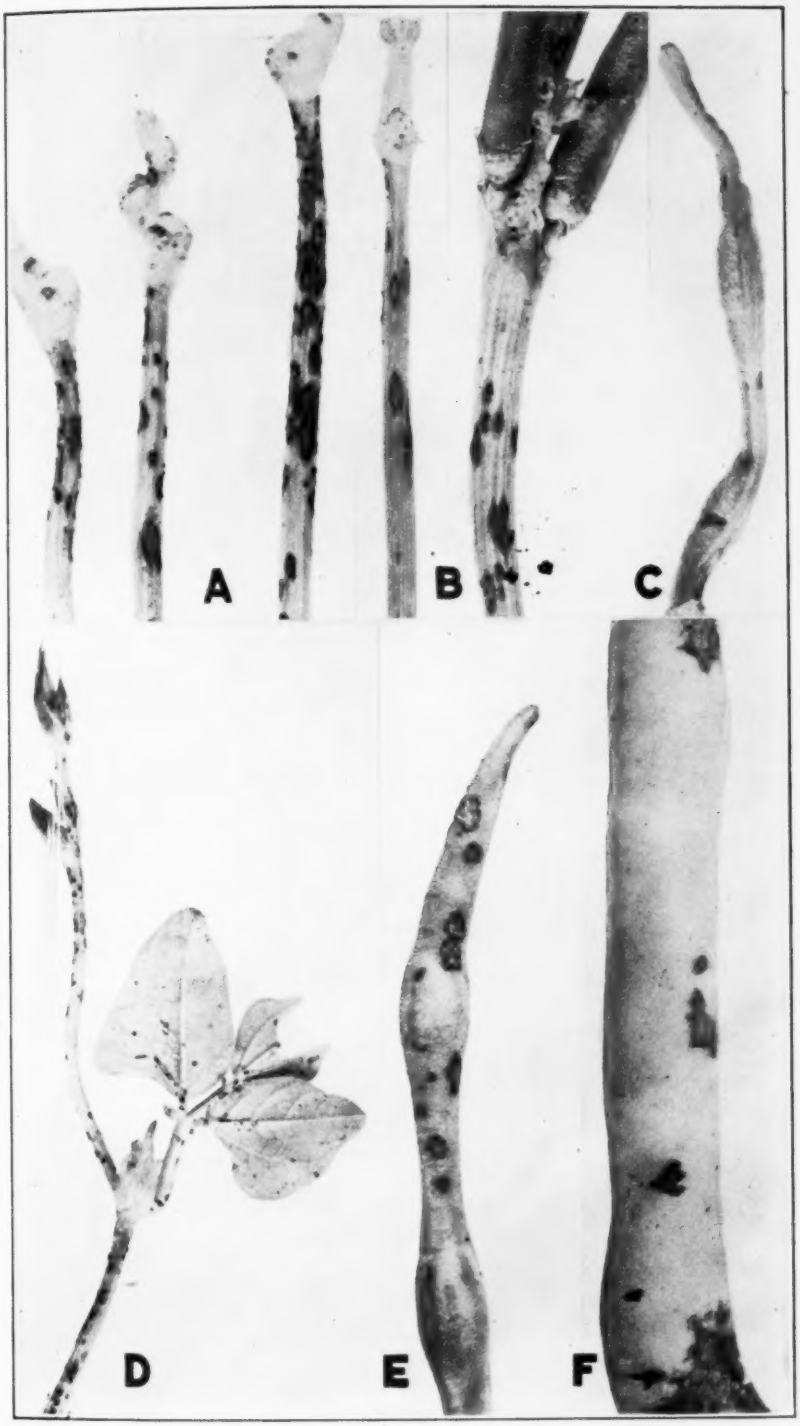
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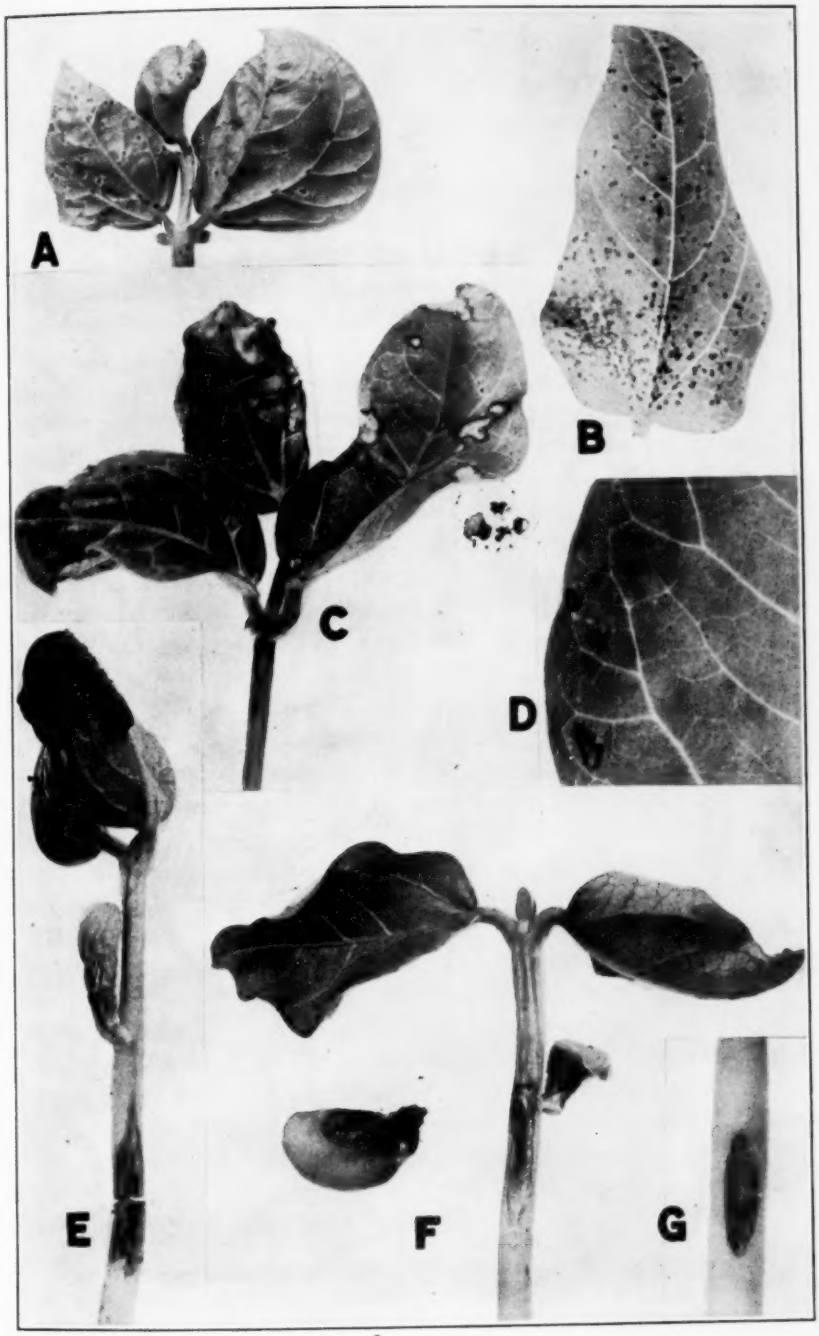
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RELATION OF GROWTH OF HELMINTHOSPORIUM SACCHARI TO MAINTAINED TEMPERATURES[1]

F. F. Halma and H. S. Fawcett

It is generally recognized that the growth of parasitic fungi is greatly influenced by temperature. Among many recent investigations which have a bearing on this question a few of the recent ones may be mentioned.

According to Dickson (1), soil temperature is the most important single factor determining the extent of seedling blight of wheat and corn. Rosenbaum and Ramsey (2) found that the development of blackleg of the potato is closely correlated with temperature and precipitation. Stevens (3) has shown that the rate of increase in diameter of cankers of chestnut blight varies with the temperature. Edson and Shapovalov (4) found a certain degree of correlation between temperature relations of some potato fungi in pure culture and their geographical distribution and seasonal occurrence. Lauritzen and Harter (5) established the fact that of the two species of *Rhizopus* responsible for soft rot in sweet potatoes one is responsible for decay at a higher and the other at a lower temperature. Walker and Jones (6) believe that the regional distribution of onion smut in the United States is conditioned by the soil temperature during the seedling stage of the plant's growth. Fawcett (7, 8) has suggested that temperature conditions may be among the most important factors limiting the prevalence of diseases due to *Phomopsis citri* Faw. and *Cladosporium citri* Mass.[2] In another investigation (9) he found that the growth of four different fungi were characteristically affected by temperature.

From the literature cited above it is evident that a knowledge of temperature relations is of importance to the investigator working with any fungus. It is for this reason that the results of this investigation are placed on record.

Mr. H. A. Lee, of the Hawaiian Sugar Planters' Association, wishing to ascertain the temperature relations of *Helminthosporium sacchari*, sent cultures to this laboratory where facilities for such an investigation are available. From these cultures several stock cultures were made by transferring small pieces of mycelium to petri dishes containing standard nutrient agar. This constituted the source of the material used.

[1] Paper No. 124, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] This fungus is being described by Miss Anna E. Jenkins as *Sphaceloma fawcettii*.

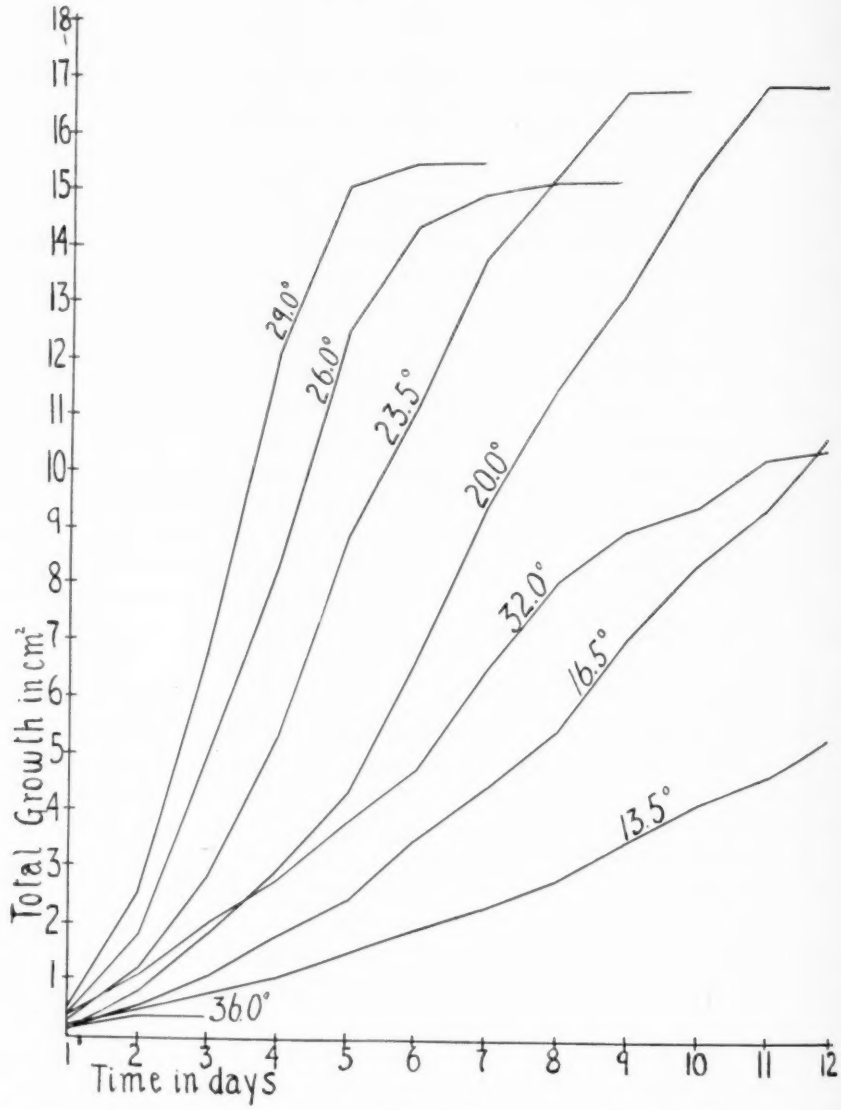

FIG. 1. Graph showing the growth of *Helminthosporium sacchari* at different temperatures with time.

The fungus was grown in standard nutrient agar and in standard bouillon. Each petri dish contained 15 cc. of the former and each Erlenmeyer flask 100 cc. of the latter. The hydrogen-ion concentration of the bouillon was pH 6.8. A disk 2 mm. in diameter, cut out near the advancing margin of the mycelium of a stock culture, was introduced into each of the petri

dishes and flasks. These were immediately placed in the various temperature chambers.

For each temperature ten petri dishes and ten flasks were used. Eight different temperatures were employed as follows: 13.5, 16.5, 20.0, 23.5, 26.0, 29.0, 32.0, and 36.0° C. These temperatures with the exception of 36° C. (which was maintained in an incubator) were maintained by means of an apparatus similar to that described by Livingston and Fawcett (10).

The increment in the diameter of the mycelial mass was measured daily. From these data the area was calculated and this was taken as the index of growth. The mycelium in the bouillon was allowed to grow for ten

TABLE 1.—*Growth of Helminthosporium sacchari on standard nutrient agar at different temperatures*

Days	Temperature in degrees Centigrade							
	13.5	16.5	20.0	23.5	26.0	29.0	32.0	36.0
1	5.1[a]	4.6	4.8	6.5	7.4	8.6	6.9	5.2
	0.2	0.2	0.2	0.3	0.4	0.6	0.4	0.2
2	7.6	8.6	10.1	12.7	15.4	18.2	12.1	7.2
	0.5	0.6	0.9	1.2	1.9	2.6	1.1	0.4
3	10.1	11.8	15.2	19.1	25.1	29.7	16.0	Growth ceased
	0.8	1.1	1.8	2.8	4.9	6.9	2.0	
4	12.1	15.2	19.2	26.1	32.6	39.2	18.8	
	1.1	1.8	2.9	5.3	8.3	12.1	2.8	
5	14.0	17.5	23.7	33.6	40.1	43.8	21.9	
	1.5	2.4	4.4	8.9	12.6	15.1	3.8	
6	15.7	21.0	29.2	37.8	42.8	44.5	24.6	
	1.9	3.5	6.7	11.2	14.4	15.5	4.8	
7	17.3	23.6	34.6	41.9	43.7	Growth ceased	28.9	
	2.3	4.4	9.4	13.8	14.9		6.5	
8	19.1	26.9	38.2	44.3	44.0		32.3	
	2.8	5.7	11.5	15.3	15.2		8.1	
9	21.1	30.1	41.1	46.3	Growth ceased		33.8	
	3.5	7.1	13.2	16.8			9.0	
10	23.1	32.6	44.2	Growth ceased			34.7	
	4.2	8.3	15.3				9.4	
11	24.5	34.6	46.4				36.3	
	4.7	9.4	16.9				10.3	
12	25.9	36.9	Growth ceased				36.5	
	5.3	10.7					10.4	

[a] Upper number = diameter of mycelium in mm.; lower number = area of mycelium in cm².

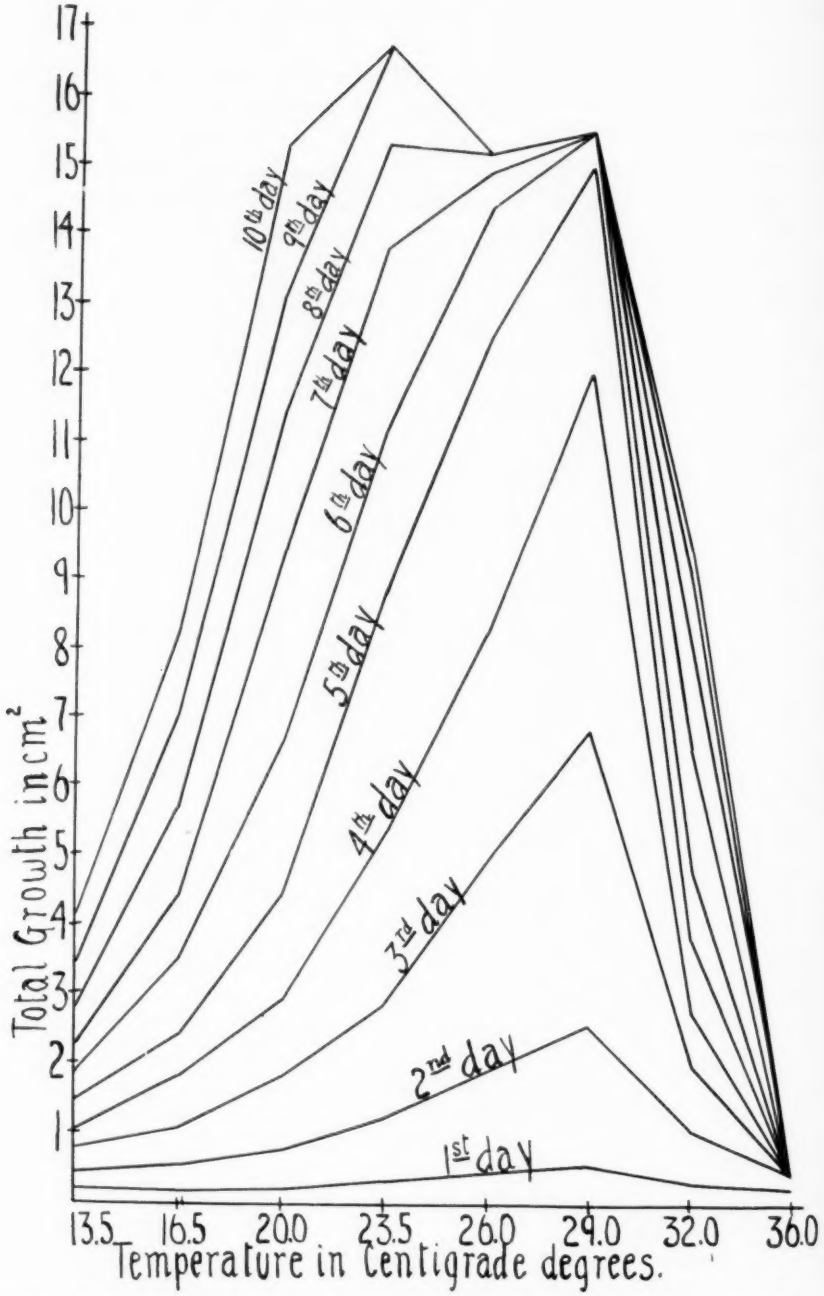

FIG. 2. Graph showing the rate of growth of *Helminthosporium sacchari* at different temperatures.

days, then filtered through "Genuine Whatman Filter Paper" which had previously been weighed. The mycelium was thoroughly washed with distilled water, then air dried in a closed room for one week. The total net weight of the air-dried mycelium of each set was taken as a measure of growth. The hydrogen-ion concentration of the bouillon both before and at the completion of the experiment was determined by comparing it with standard buffer solutions.

GROWTH IN STANDARD NUTRIENT AGAR

Table 1 and figures 1 and 2 show that, with the exception of 36° C., the fungus was able to grow in all the temperatures employed. It may be

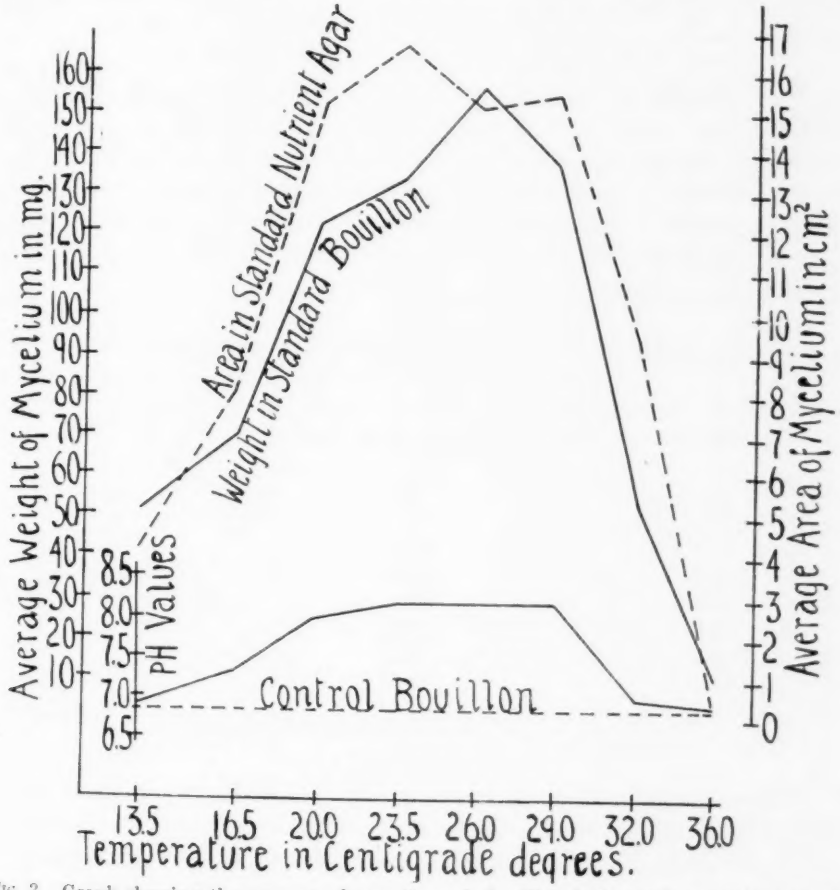

FIG. 3. Graph showing the amount of growth made by *Helminthosporium sacchari* in 10 days in solid and liquid media and the change in the hydrogen-ion concentration of the bouillon.

mentioned in this connection that the organism was not destroyed at 36° C., as growth took place promptly when the petri dishes were transferred to room temperature after remaining at 36° C. for ten days.

Under the condition of the experiment the optimum temperature for *Helminthosporium sacchari* is seen to lie between 20° and 29° C. However, the most rapid growth took place at 29° C. until it came to a stop on the seventh day; then the optimum gradually shifted to the lower temperatures until the twelfth day, when growth ceased in the optimum range. It is of interest to note that in all the four temperatures lying between 20° and 29° C. the final average area of the mycelium was practically the same. In no case did the mycelium occupy more than one-third of the area of the medium.

GROWTH IN STANDARD BOUILLON

The data given in table 2 and figure 3 confirm the results obtained in nutrient agar, namely, that the optimum range for this fungus lies between 20° and 29° C. An additional point of interest is that the reaction of the bouillon changed with the amount of growth. The pH value of the bouillon remained practically the same at temperatures at which only slight growth took place; but in the optimum range the bouillon became distinctly alkaline (see table 2).

TABLE 2.—*Growth of Helminthosporium sacchari in standard bouillon at different temperatures*

Temp. C°	Average wt. of air-dried mycelium in mg.	pH of bouillon at end of 10th day
13.5	51.0	6.9
16.5	75.0	7.3
20.0	126.0	8.0
23.5	132.5	8.2
26.0	157.0	8.2
29.0	136.5	8.2
32.0	53.5	7.0
36.0	10.0	6.9

SUMMARY

Helminthosporium sacchari was grown in standard nutrient agar and standard bouillon at eight different temperatures. Under the conditions of the experiment the optimum temperature for this fungus was found to lie between 20° and 29° C. The optimum, however, in nutrient agar remained constantly at 29° C. for the first seven days, when growth ceased

at that temperature. Within the optimum range (20° to 29° C.) the reaction of the bouillon became distinctly alkaline, whereas only a slight change in pH occurred at those temperatures at which the growth was slight.

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HIGH EVAPORATION: A PRECURSOR AND A CONCOMITANT OF WESTERN YELLOW TOMATO BLIGHT

MICHAEL SHAPOVALOV

WITH SIX FIGURES IN THE TEXT

IT is generally recognized that climatic conditions may either favor or arrest the development of plant diseases, but not all climatic factors involved have received the recognition which they deserve. As bacterial and fungus parasites are the principal causative agents of the diseases, a humid atmosphere accompanied by a medium to high temperature appears to be most favorable for the development of many troubles. Consequently, the attention of investigators was mainly given to temperature, precipitation, and higher humidities. The rôle of evaporation, especially of high rates of evaporation, has been decidedly less studied and less understood. However, the connection between the rate of evaporation and certain plant diseases is apparent and the need of more complete evaporation data for various agricultural sections is already being felt.

In the case of western yellow tomato blight,[1] a certain seasonal march of evaporation means a definite progress of blight in the same season. This disease causes serious annual losses to the growers over a large territory extending west of the Rocky Mountains to the Pacific coast and from British Columbia to the west coast of Mexico, but the actual annual damage fluctuates according to seasonal conditions. The summer of 1924 was marked by a particularly severe outbreak of western blight in a number of widely separated regions of the West and for this reason is especially interesting. This outbreak correlates with an unusually high evaporation in all those sections in which it occurred, as will be seen from the following analysis.

Reports from Utah show that the damage from blight in that state last summer was the most serious since the epidemic of 1905 and by far exceeded the average annual damage from the disease in the state. It is generally estimated that the last year's loss ranged between 30 and 35 per cent of the state crop, while the average annual loss probably does not exceed 5 per cent.[2] The toll taken by this blight in the state of Washington was even

[1] The term "blight" is frequently used in this paper for the sake of brevity, but in every instance it has reference to western yellow blight, a disease characterized by a general yellowing and a pronounced rigidity of the plant, torsion of the leaves, purpling of the veins and cessation of growth, followed by death and accompanied by a progressive root decay.

[2] Plant Disease Reporter 8: nos. 6 and 10. U. S. Department of Agriculture, Washington, D. C. 1924.

more severe. It reached an average of probably 50 per cent, and many patches were observed where 75 per cent of the plants were affected.[3] Similar conditions also prevailed in the southwestern corner of Idaho where climatic conditions are identical with those of the adjacent portions of Washington. Tomato-growing in California is scattered in climatically different and even contrasting regions, and the amount of the disease in these regions varied from practically nothing in humid sections near the coast to nearly 100 per cent in localities with a high rate of evaporation. The severest attack of the disease in the state last summer occurred at Shafter (near Bakersfield) where, in a field of 1700 plants, all but 16 died from blight by the end of June, and only 3 were left unaffected by the end of July. Also quite severe loss was suffered by tomato growers in the section surrounding Riverside. Nearly 40 per cent of plants blighted in 1924 in the writer's experimental field at Riverside, in which less than 2 per cent blighted in 1923, the same variety (Stone) being used both years. Finally, it is very significant that the first definite report of the occurrence of western tomato blight east of the Rocky Mountains, in the state of Kansas,[4] was received in 1924. The disease did not appear to be very severe there, as only a few plants here and there in a patch blighted, but it was scattered in various parts of the state. Heretofore the reports were practically confined to the territory west of the Rocky Mountains, with a few cases reported from as far as New Mexico.[5]

It is very illuminating to compare these reports of the unusually severe outbreaks of the tomato blight with the weather conditions in certain representative sections of the states just mentioned. It will be seen (Figs. 1–4)[6] that the rate of evaporation in 1924 was in every case above the average of a number of preceding years. This condition was due mainly to a lower relative humidity and a greater wind movement. The relative humidity at Salt Lake City in 1905 was much below the average, indicating a high evaporation, which explains the fact that that year is remembered in the state as a severe blight year. The departures of mean temperatures are not significant and not uniform for various blight sections. The same may be said of the precipitation means for Utah and Washington; but in Wichita, Kans.,

[3] Plant Disease Reporter **8**: no. 10. U. S. Department of Agriculture, Washington, D. C. 1924.

[4] Reported by Dr. L. E. Melchers, of the Kansas State Agricultural College.

[5] Plant Disease Reporter **8**: no. 6. U. S. Department of Agriculture, Washington, D. C. 1924.

[6] The data for all figures except figure 4 were obtained from the U. S. Weather Bureau publications, the Monthly Climatological Data and the Reports of the Chief. The evaporation data for figure 4 were secured by the writer by means of porous porcelain cup atmometers.

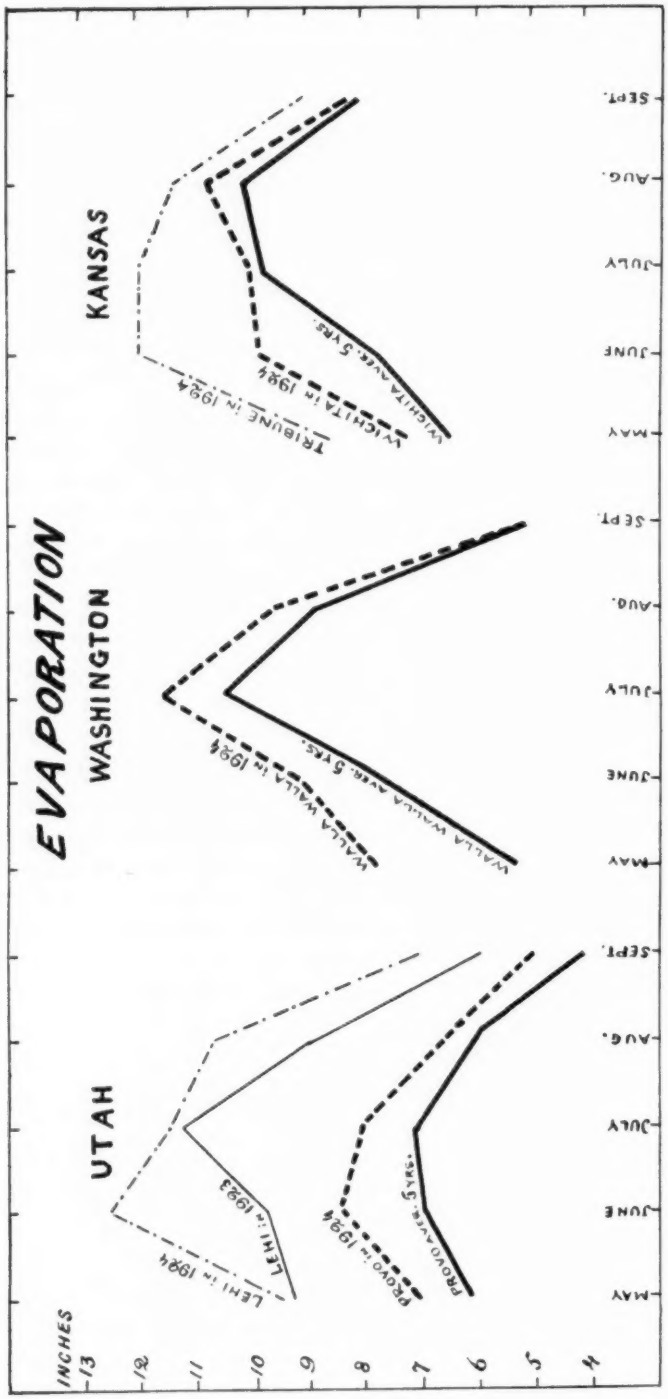

FIG. 1. Showing an excessive rate of evaporation in 1924 in some of the sections from which outbreaks of western yellow blight were reported.

it not only fell considerably below the normal during the month of June, but even reached the level of arid sections of Utah and Washington. This extraordinary deficiency in rainfall at Wichita (and other tomato-growing sections of the state as well) may have played an important rôle, emphasizing the effect of the conditions causing high evaporation and thus making the occurrence of western yellow blight in the state possible.

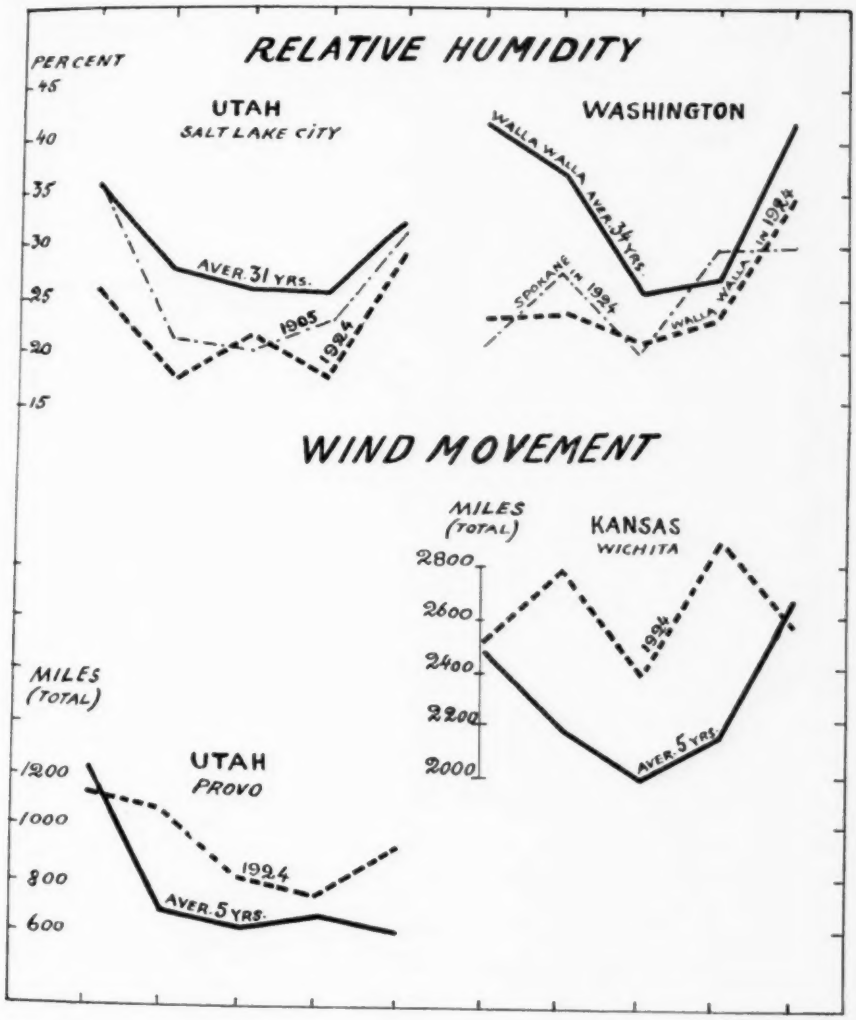

FIG. 2. Showing unusually low relative humidity and high wind movement in 1924 in several localities where western yellow blight was particularly severe.

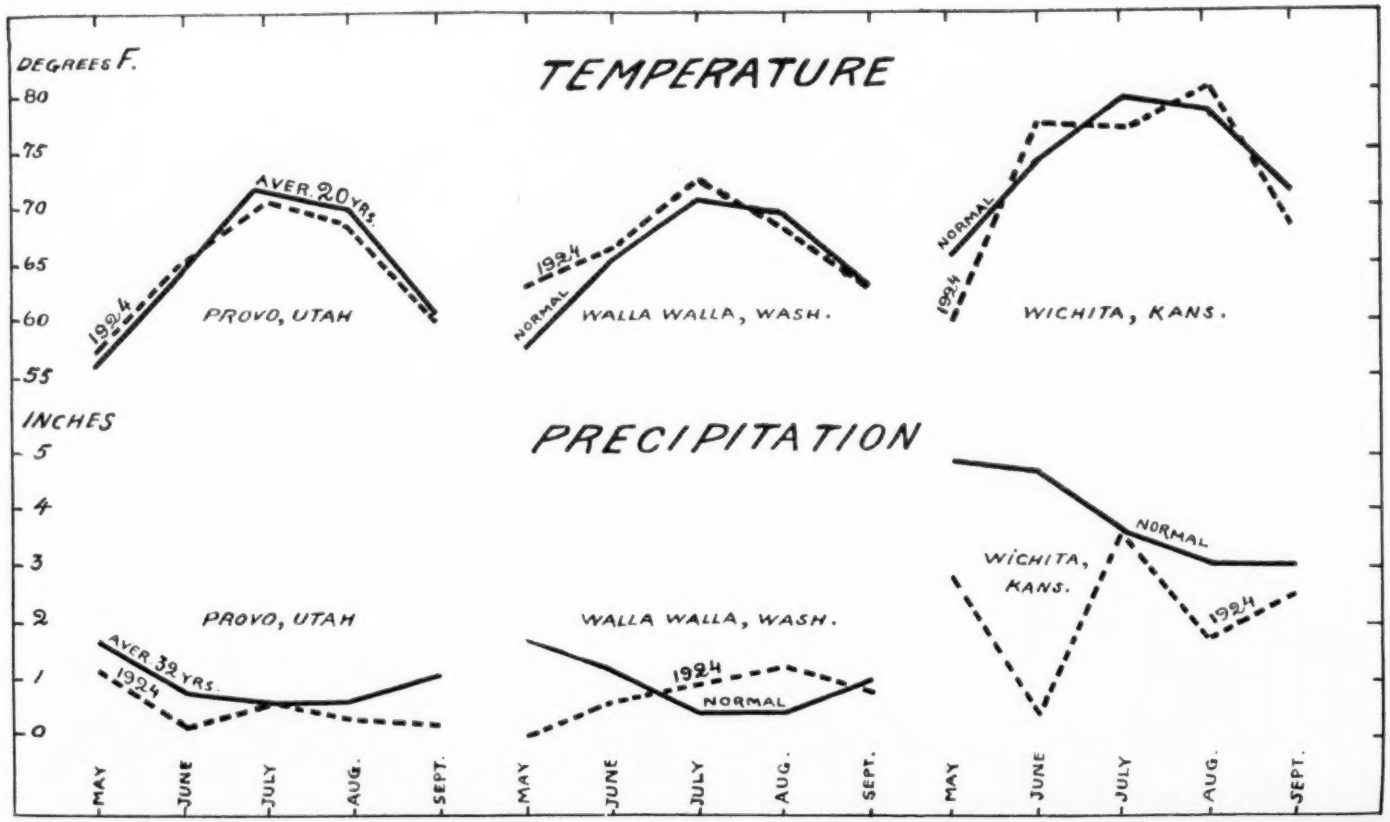

FIG. 3. Showing temperature and precipitation in certain blight sections in 1924 as compared with averages and normals. It can be seen that monthly fluctuations are not uniform and do not correlate with the progress of the disease.

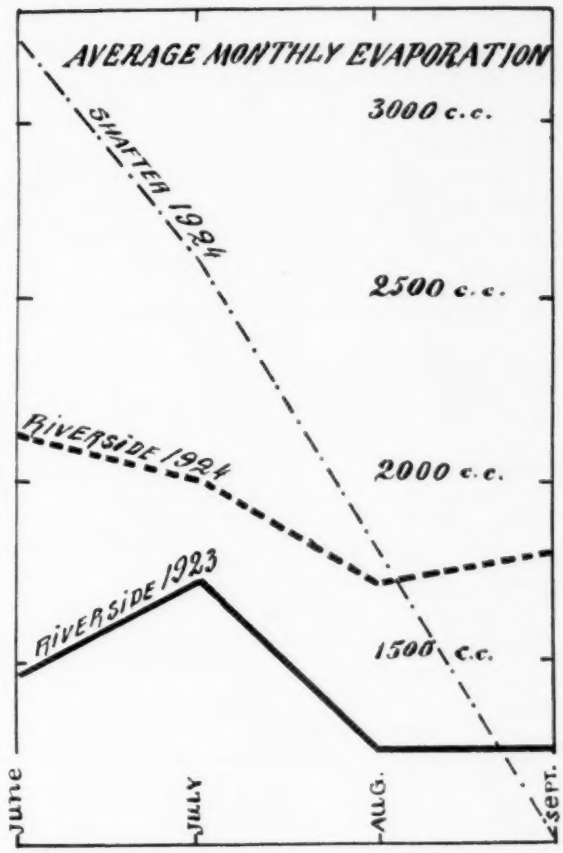

FIG. 4. Evaporation on experiment plots at Shafter, Calif., and Riverside, Calif., as related to the progress of western yellow blight. There was 1.7 percent of blight at Riverside in 1923, 39.1 percent in 1924, and practically 100.0 percent at Shafter in 1924.

The most striking correlation between the amount of blight and the rate of evaporation was observed on the experimental plots at Shafter, Cal., and at Riverside, Cal., where counts of blighted plants were made throughout the season. The disease developed slowly and in very small amounts with the lowest evaporation curve at Riverside in 1923. It was more severe in 1924 when the evaporation was higher, but the most serious attack and in much shorter time developed with the highest rate of evaporation at Shafter in 1924.

The comparison of the percentages of blight at Riverside and at Shafter in 1924 indicates that the rate of evaporation is not only concomitant with the severity of blight in different seasons in the same locality, but also cor-

FIG. 3. Showing temperature and precipitation in certain blight sections in 1924 as compared with averages and normals. It can be seen that monthly fluctuations are not uniform and do not correlate with the progress of the disease.

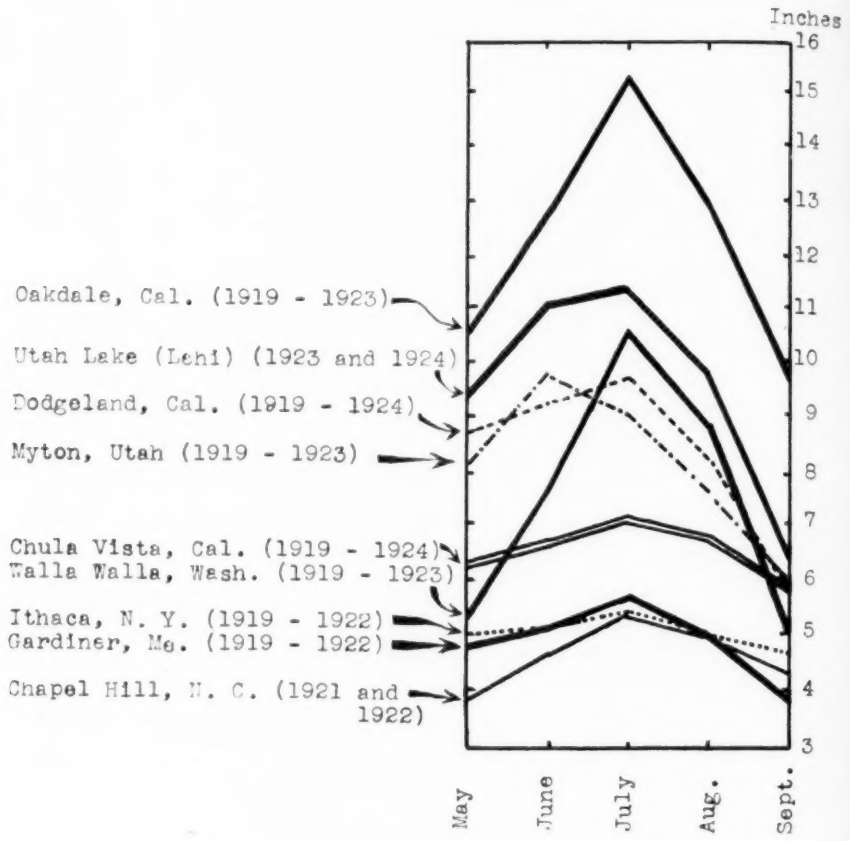

FIG. 5. Evaporation (average monthly amounts).

relates with its geographical distribution. This may be illustrated by comparing various blight and non-blight sections of the West. The blight problem is more or less serious south of Lake Okanagan and in the state of Washington, through the valleys of Wenatchee and Yakima, and in the Clarkston-Lewiston district on the Snake River, also at The Dalles, Ore., in the territory east of the Great Salt Lake of Utah, in the Sacramento, San Joaquin and San Fernando Valleys of California, near Riverside, near Sinaloa (Los Mochis), and Culiacan, Sin., Mexico, and, in general, in the western irrigated territory. It is of slight or no importance near Vancouver, at Puget Sound, in the vicinity of Portland, in the Bay Region of San Francisco, in the coastal trucking sections of Los Angeles, Orange, and San Diego counties, near Mazatlan, Sin., Mexico, and, in general, in the eastern United States. Figures 5 and 6 give a comparison of the rates of evaporation and the rela-

tive humidity of these blight-free and blight-infested geographical regions. The greatest difference occurs during the summer months of June, July, and August, which are also the principal blight months. There is a regrettable scarcity of localities (especially within the agricultural territory) for which evaporation records are taken. Out of nine such localities (given in Fig. 5) lying in or near tomato sections, five are blight sections and have the average monthly evaporation during the blight period above 7 inches, and the remaining four are blight-free sections with the average monthly evaporation for the same period at or below 7 inches (Chula Vista being at the top of this group). Relative humidity records are available for a greater num-

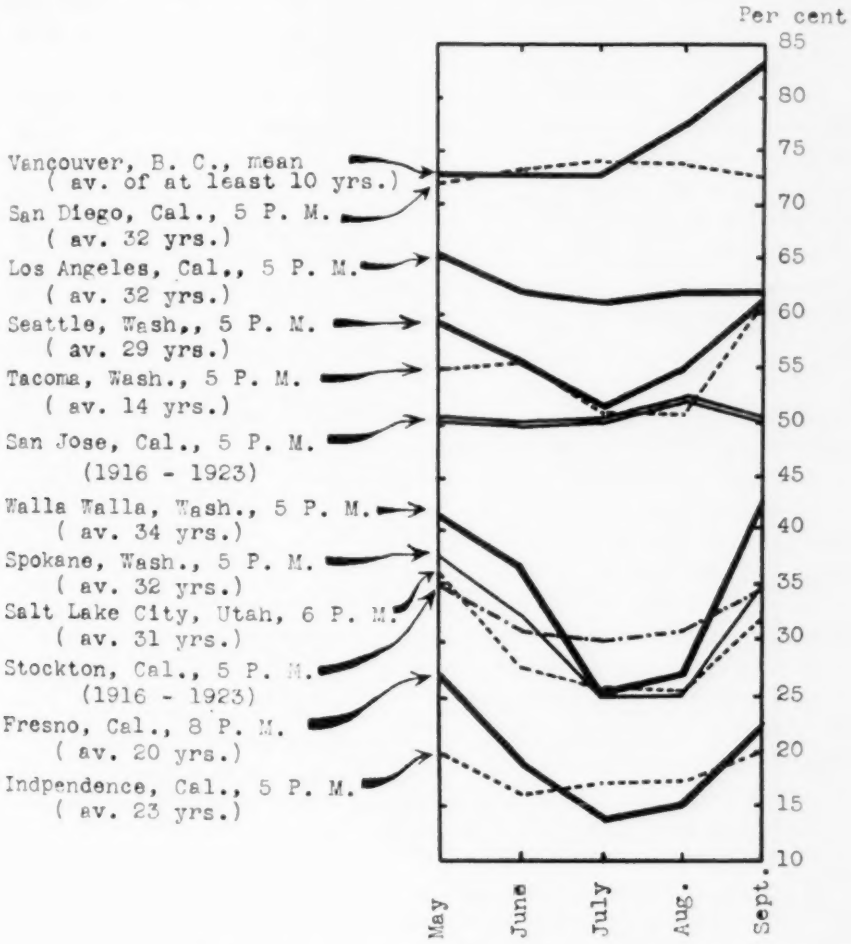

FIG. 6. Mean relative humidity (averages).

ber of stations, and figure 6 gives the humidity curves for six from each of the two blight-contrasting groups of localities. Blight areas during the principal blight period seem to have the average monthly humidity below 35 per cent, while non-blight areas about or above 50 per cent. (San Jose, which is a moderate blight section, approaches this latter group during the second part of the summer.) The extremes for each group are to be found in the San Joaquin valley for the former, and near Vancouver and San Diego for the latter. High evaporation is not only attendant on, but also foreruns severe spells of blight, especially at the outset of the season. This was found to be true in 1924 both at Riverside and at Shafter. Detailed weekly observations at these places from the time the plants were set out in the field show very distinctly that the first serious waves of the disease were preceded by marked rises in the rate of evaporation.

Not only the progress of blight is arrested, but a phenomenon of an entirely different order takes place when the evaporation drops to a low level for a more or less protracted period. In this case such blighted plants as are not in the last stage of the disease show a recovery which, although ordinarily not complete and seldom leading to the restoration of the normal state, is nevertheless very significant scientifically. On the Riverside experimental plots in 1924 nearly 10 per cent of the total number of blighted plants showed partial recovery (one plant recovered completely) during the month of August, when the evaporation was at its lowest ebb for the four summer months.

It is thus quite apparent that the evaporation data are indispensable for the conduct of western yellow tomato blight studies as well as for a better understanding of the normal growth of the tomato plant, as the conditions which favor high rate of evaporation also favor severe outbreaks of this blight. This was exceedingly well illustrated by the condition which prevailed during the 1924 season.

It is most likely that the evaporation will be found to have an important bearing on the disease problem of many agricultural crops when ecological relationships of these latter are better understood. Adequate evaporation records would both facilitate such understanding and stimulate new efforts towards it. Consequently, it is very urgent that more weather stations, especially those located in agricultural sections, gather evaporation data along with the data on relative humidity, wind movement, sunshine, and other factors influencing the rate of evaporation.

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FUMIGATION INJURY OF WATERMELONS

G. B. RAMSEY[1]

WITH ONE FIGURE IN THE TEXT

During the past season, the Food Products Inspectors of the Bureau of Agricultural Economics were called upon to inspect several cars of California watermelons for a peculiar type of injury that was causing extensive damage in certain cars. The writer's attention was first called to this trouble by Mr. R. L. Ringer, of Portland, Oregon. At one time he inspected five cars in which every melon was blemished by blisters and sunken areas which resembled anthracnose, but upon inquiry of the authorities at place of origin it was found that neither vines nor fruits in the field showed evidence of anthracnose. The question was then raised as to whether the fumigation with formaldehyde, as required by the quarantine on account of the "Hoof and Mouth" disease, might not be responsible for this damage. Shipments into the state of Washington were required to be fumigated, but Oregon did not require fumigation, and this fact led to a striking demonstration which points directly to the formaldehyde fumes as the damaging agent. A stock car of melons arrived in Oregon in perfect condition, but before being sent on into Washington, they had to be fumigated. The melons were therefore transferred to a refrigerator car that could be closed up tightly and were fumigated and sent on their way to Vancouver, Wash. On arrival, forty-eight hours later, every melon was blistered and pitted. Over fifty cars of California watermelons are known to have been seriously affected by this type of injury.

Mr. B. A. Harrigan, Horticultural Commissioner at El Centro, California, writes regarding this trouble:

> The only places we experienced pitting and sunken areas were in melons that sat in the sun too long after having been picked. We found that melons picked during midday and left in the field would show considerable damage in five hours. Judging from past experience I believe that watermelons will show no damage, from fumigation, if they are picked before ten o'clock in the morning and after four o'clock in the afternoon and moved out of the field.

[1] Contribution from the Research Laboratory on Market Diseases of Vegetables and Fruits; United States Department of Agriculture, Bureau of Plant Industry, and the Botany Department, University of Chicago, cooperating.

The writer has seen similar lesions caused by fumigation, in other products, so it seemed not unreasonable to think that this particular watermelon trouble might be associated with fumigation. With a view to testing the possibility of reproducing this injury, a perfectly smooth and well-colored melon was placed in a closed transfer chamber and fumigated with formaldehyde. The formula used was the same as that recommended for the car-lot treatment of fruits and vegetables for control of the "Hoof and Mouth" disease, viz.: potassium permanganate 16 ozs., formaldehyde 20 ozs., per 1,000 cu. ft. of air space, for a period of four hours. The glass walls of the transfer chamber permitted the observer to see the melon without liberating any of the fumes. At the end of the four-hour period, no changes were noted in the melon. The door of the transfer chamber was then opened momentarily in order to detect and determine to some degree the strength of the fumes by the odor. The chamber was immediately closed and not opened again for fourteen hours. At the end of

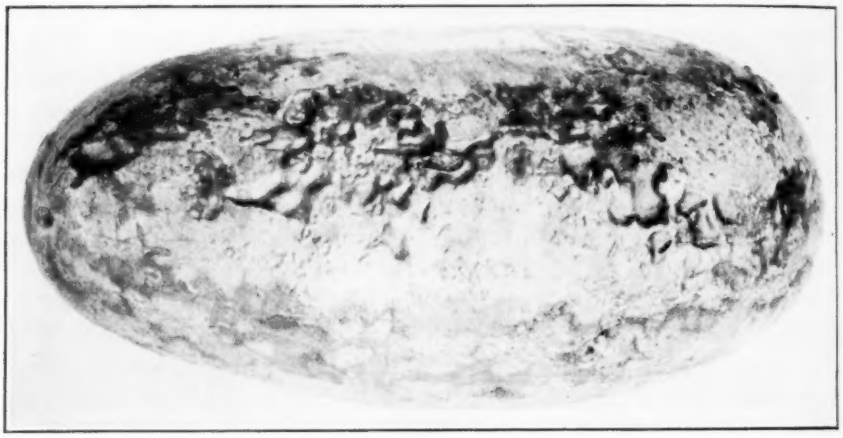

FIG. 1. Fumigation injury.

this time no injury was apparent, but the door was opened and the remaining fumes allowed to escape. It was not until approximately twenty-seven hours later that definite pits became evident. At this time several small depressions from one-eighth to one-fourth inch in diameter and a few larger ones from one-fourth to one-half inch in diameter were scattered irregularly over the fruit. The larger and deeper lesions were in regions where the melon was slightly bruised or where the cuticle had been rubbed off. These bruises, however, were not apparent as discolorations or depressed areas at the beginning of the experiment.

This experiment clearly demonstrates that lesions of the type shown in figure 1 can be produced by formaldehyde fumigation. The fact that not all fumigated cars showed injury indicates that a careful study of the technique of application with due attention to environmental factors may be expected to indicate a procedure which is both safe and effective should occasion again arise for fumigating watermelons.

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PYTHIUM INFECTION OF CABBAGE HEADS

CHARLES DRECHSLER

WITH ONE FIGURE IN THE TEXT

In a previous paper[1] dealing with the cottony leak of cucumbers were enumerated some recorded instances in which members of the genus *Pythium* have been found responsible for destruction to plant products. Such cases are deserving of special note, inasmuch as they differ from the instances of damage from damping off and rootlet injury more usually associated with the genus, in that they involve tissues of relatively mature parts. Moreover in certain cases where destruction may be initiated or continued after harvesting, the control problem presented is a different one, consisting, as it does, in the conservation of plant structures under conditions more or less subject to control, rather than in maintenance of the life of the plant or its efficiency as a productive unit by the somewhat indirect and not always effectual means of soil management.

To the instances enumerated may be added a type of decay of cabbage not hitherto described. In July, 1924, the writer received for identification a portion of a cabbage head found on the Washington market by Miss L. McCulloch. Some of the compactly arranged inner leaves were conspicuously water-soaked in appearance, the discolored regions extending somewhat farther along the fleshy midribs than over the thinner lamellae, and evidently proceeding from the base where the modified foliar organs were attached to the affected core. When the affected leaves were separated out, the diseased portions were found to be nearly as firm to the touch as the healthy portions. That this firmness was due mainly to the surface tissues became apparent on dissection, by which means the deeper tissues were revealed as a softened pulpy mass from which water could be freely expressed.

When examined under the microscope the pulpy material was found to consist very largely of abundantly branching, continuous mycelium of the type characteristic of *Pythium* when developing in culture media rich in organic matter, or, for example, in living cucumber or watermelon fruits. And as in these substrata the tissue structures of the host were reduced to disorganized cell walls small in quantity when compared to the abundance of mycelial development. Portions of the material planted on cornmeal agar yielded cultures of a species of *Pythium*, which subsequently were freed of contaminating bacteria.

[1] Drechsler, Charles. The cottony leak of cucumbers caused by *Pythium aphanidermatum*. In press; to appear in the Journal of Agricultural Research.

A fuller treatment of the morphology and taxonomy of the fungus is reserved for inclusion in a comparative account of the genus *Pythium* now under preparation. It would appear to be a species of the type usually dealt with in the literature as *Pythium debaryanum* Hesse producing subspherical "conidia," smooth oogonia, and oospores, and on suitable artificial media an abundance of cottony aerial mycelium. Hesse's binomial, however, cannot appropriately be applied to it as the sexual apparatus shows marked departures from that distinctive of *Pythium debaryanum*, particularly in the relationship of antheridium to oogonium. When the oogonium is intercalary and borne on the larger hyphae, fertilization is generally accomplished by cylindrical stalked antheridia, of which one or two are usually present, communication being established by a tube entering directly from the septum originally delimiting the two organs. When more delicate hyphae are involved, more frequently a sessile pouch-like outgrowth develops immediately adjacent to the oogonium, and this together with a variable but usually small portion of the hypha is delimited by a septum and functions as an antheridium, communication being established by a tube originating from the pouch-like part and traversing the oogonial wall a short distance from its juncture with the parent filament. Not infrequently antheridia developing from a hypha other than the one bearing the oogonium are encountered, these being either of the "branch" type with the septum at the base of an inflated part, or of a modified "intercalary" type consisting of an intercalary portion of hypha bearing a sessile inflated protuberance from which the fertilization tube is produced, the delimiting septa then being two in number and inserted directly in the hypha. The condition figured by Hesse, and made familiar by numerous text-book illustrations, of fertilization being accomplished by an approximately cylindrical or slightly swollen antheridium terminating a branch arising some distance below the oogonium from the same hypha as the latter, has not been observed.

The pathogenicity of the fungus to cabbage was established by inoculating healthy heads through incisions at the base of the stalk. Extensive water soaking in the region surrounding the inoculum became evident within 24 hours, the tissues of the core simultaneously becoming softened to a narrow-like consistency. Eight days after inoculation, on cutting the specimens, the condition shown in figure 1 was found: the core entirely softened, and the infection extending into all the foliar elements that make up the head for distances up to 60 millimeters. Although some mycelium was present in the spaces between the leaves, the fungus appeared to progress largely within the individual leaves, attaining its greatest development, as in the original specimen, in the tissues of the thickened fleshy

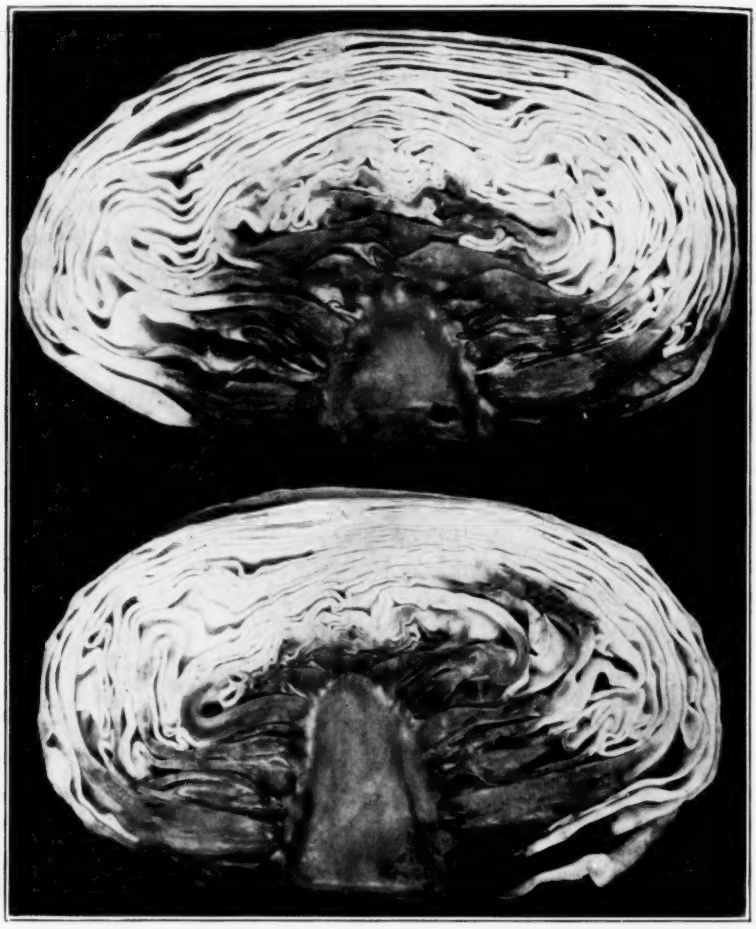

FIGURE 1. Two cabbage heads 8 days after inoculation at the base of the stump with a pure culture of the *Pythium* species isolated from a naturally infected head. Enlarged × ½.

midrib. Here a watery condition obtained not greatly unlike that which suggested the terms "leak" and "cottony leak" for diseases caused by congeneric forms in potatoes and cucumbers respectively, although, owing to preservation of the outer layers of tissue, the liquid is usually retained, so that the head, as a whole, does not become markedly wet. A mild, not unpleasant odor, as of stewing cabbage, appears to be produced by the activity of the parasite.

Concerning the prevalence of the disease in question nothing definite is known. The writer is informed that a type of damage quite similar to

that resulting from artificial inoculations has been observed on cabbage in the New York market, where it is usually known among the trade as "stump-rot" and generally assumed to be a form of bacterial soft rot. It should be mentioned that as in other cases of Pythium infection, after the cabbage tissues have been killed, bacteria multiply, so that the parts take on the texture and emit the disagreeable odor of putrifying material. It is hoped that further observation may yield information concerning the prevalence of Pythium infection and its possible relation to stump-rot.

Inoculation experiments carried out on cabbage heads, using various species of *Pythium*, indicate, as might be expected from experience in parallel cases, that pathogenicity is not confined to the form isolated from the host in question. A number of species of the "debaryanum" type derived from various sources, as, for example, from potatoes affected with leak, from diseased pea roots, from diseased sweet-potato rootlets, from sweet-potato roots affected with mottle necrosis, and from watermelon fruit affected with blossom-end rot, produced similar pathological effects, in some instances more rapidly, in other instances less rapidly than the cabbage form. Strains of *P. aphanidermatum* (Edson) Fitz. isolated from diseased watermelon fruit and from cucumbers affected with cottony leak, also gave positive results. On the other hand, certain forms of the "debaryanum" type gave only negative results. None of the species with spiny oogonia appear to be capable of infecting cabbage, nor have any of the forms with a similar intramatrical habit but with smooth oogonia, among which *P. monospermum* Pringsheim could be definitely identified, shown any indication of pathogenicity.

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FUSARIUM VASINFECTUM AND THE DAMPING OFF OF COTTON SEEDLINGS

H. R. Rosen

During May, 1923, a very poor stand of cotton seedlings was noted in certain field plots in contrast to others in which the seed used had been the same and which showed a far superior stand. These plots had been artificially infested with pure cultures of the cotton-wilt fungus. In order to insure thorough infestation, the fungus growing on bran was heavily applied in open furrows, and the cotton seed was sown by hand directly upon the inoculated bran. Within a few days after planting and before the plants had appeared, a heavy fungus growth could be clearly seen across the field, marking the paths where the cultures had been applied. Considerable rain fell and there was very little sunshine during the period.

A field approximately one acre in extent had been used, and in cooperation with the Agronomy Department, University of Arkansas, the field had been divided into six sections and different fertilizers had been applied to one-half of each section. On three of these sections the unfertilized parts showed a very much poorer stand than upon the fertilized, and when seeds that had failed to produce plants were dug up it was found that some of them had apparently begun to sprout and had then been killed, while others had died apparently before the emergence of radicle or hypocotyl from the seed coats. A number appeared as seedlings just emerging from the hull, showing dark discolored and rotted parts of the hypocotyl, and without sufficient vigor to raise the plumule above ground. Also, of the seedlings that had pushed above the soil, quite a number showed typical symptoms of damping off and sore shin. From several such seedlings which had received surface sterilization, pure cultures of *Fusarium vasinfectum* were obtained, as ascertained by the color reaction on rice[1] and by inoculating sterilized soil with cultures and noting wilt development on plants grown in such soil.

It was of considerable interest to see that the stand was not equally superior on all fertilized, compared to unfertilized, plots. The 3 one-tenth acre plots receiving kainit (40 lbs.), muriate of potash (12 lbs.), and Utah potash (12 lbs.), respectively, showed greater stand, as compared to the unfertilized plots, than did any of the 3 following combinations: (1) acid phosphate (34 lbs.), nitrate of soda (12 lbs.), muriate of potash (4 lbs.); (2) acid phosphate (34 lbs.), nitrate of soda (12 lbs.), and

[1] Elliott, John A. Cotton wilt, a seed borne disease. Jour. Agr. Res. 23: 387-393. 1923.

muriate of potash (8 lbs.); and (3) acid phosphate (34 lbs.), nitrate of soda (12 lbs.), and Utah potash (8 lbs.). In all cases the fertilizer had been applied by hand in the furrows immediately on the bran cultures of the fungus.

There are two possible explanations for the greater stand on three of these fertilized plots: first, that the fertilizer having been in direct contact with the fungus may have considerably retarded its growth, or even killed it in those spots where the concentration of the dissolved salt would be considerable; second, that certain fertilizers exercise a stimulating effect on the germination of the seed or upon the growth of the seedling and enable it to withstand the attacks of this fungus.

As the relationship of the wilt-producing fungus to lack of stand, and particularly to damping off, was not considered clearly established in this field experiment, other tests were undertaken in the laboratory and in the greenhouse. (It is quite evident that under field conditions, with many uncontrolled and unknown factors, particularly soil floras, direct causes for lack of stand, etc., may be obscured.) Cotton seeds thoroughly delinted with concentrated sulphuric acid were planted in pots containing sterilized soil (pots of soil autoclaved for from two to two and a half hours at about 15 pounds pressure). As far as known, these seeds had come from healthy plants. They appeared sound and gave a high percentage of germination. The seeds having been thoroughly rinsed and dried after treatment with sulphuric acid were then soaked in a heavy spore suspension of *Fusarium vasinfectum*. Within two weeks after seeding 12 seedlings out of 24 which had shown dark discolored rotted tissue, usually around the soil line, had died of damping off; and in another pot, which had perhaps received greater amounts of water, most of the seed failed to germinate, or rather failed to appear above ground. Four died of damping off shortly after the cotyledons were free from the seed coats. In most cases the dead or dying plants were given a thorough microscopic examination, and in every instance the Fusarium fungus was identified. Numerous platings were made from attacked tissues, after the surfaces had been sterilized, and pure cultures of *Fusarium vasinfectum* obtained. On some plates, various bacteria were sometimes obtained along with Fusarium, but these were comparatively few. In no instance was Pythium or Rhizoctonia isolated, and the bacteria, mostly in the form of white colonies, were presumably saprophytes which had followed up the attacks of Fusarium. Likewise, the few deep yellow bacterial colonies which had developed around the plated tissue were very probably also saprophytes. *Pseudomonas malvacearum* Smith has often been cultured by the writer and its light yellow color on most media quite readily distinguishes it from yellowish saprophytes which may be present.

The above experiment was repeated with quite comparable results. Again seedlings with pronounced symptoms of collar rot and with Fusarium sporulating on the rotted part were surface sterilized and when plated out yielded cultures of only one fungus.

In still another series of tests, the sterilized pots were heavily inoculated with the wilt-producing fungus growing on cotton stems, and then seeded with acid delinted seed. Sore shin and damping off were again noted.

It is rather difficult to distinguish the damping off caused by Rhizoctonia from that caused by Fusarium. The writer has assumed, in common with other pathologists, that most of the damping off of cotton was due to Rhizoctonia, but, while this may be true, there is a strong possibility that *Fusarium vasinfectum* plays an important part in causing damping off, particularly in wilt-infested fields. There is not much difference in symptoms, as almost any kind of injury to growing cotton is likely to cause the reddish-brown or purplish coloring which is peculiar to cotton. However, in the case of damping off caused by Fusarium, the rotted part is usually dark in color, almost blackish, while that of Rhizoctonia is usually lighter—reddish wine-colored, or purplish. In addition to this, the darkened, rotted tissue, which is usually localized around the crown in this form of disease, has occasionally been found to extend upward through the xylem, beyond the rotted collar, comparable to the normal, darkened interior of wilted plants. Owing to the very rapid growth of Rhizoctonia on various nutrient media, particularly potato dextrose agar, it is very easy to obtain cultures of it if it is present in the diseased tissue. At about 28° C. it will outgrow most saprophytes, including bacteria as well as fungi. It is a much more vigorous grower than *F. vasinfectum* under conditions which are favorable to both.

Fusarium vasinfectum, like other vascular Fusaria, undoubtedly attacks through the roots and in common with these may be expected to do the most damage at rather high soil and air temperatures.[2] This would suggest that damping off, as well as wilting, can be expected when the weather is rather warm, whereas in the case of damping off caused by Rhizoctonia we may expect it to be worse during cool, damp weather.

Briefly summarizing—field observations, as well as laboratory and greenhouse tests involving pure culture inoculations, clearly indicate that *Fusarium vasinfectum* may prevent cotton seed from germinating properly and may attack and kill young seedlings in a manner quite comparable to damping off caused by other fungi and bacteria.

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[2] Jones, L. R. The relation of environment to disease in plants. Amer. Jour. Bot. **11**: 601–609. 1924.

NOTES ON THE PARASITISM OF ENDOTHIA GYROSA (SCHW.) FR.

JAMES R. WEIR

WITH ONE FIGURE IN THE TEXT

During the summer of 1903, the writer began a series of inoculations with a number of forest fungi in a hardwood forest near Scottsburg, Indiana. The results obtained with *Endothia gyrosa* (Schw.) Fr. found on the roots of *Quercus velutina* are of interest since they corroborate the work of Shear, Stevens,[1] and Clinton,[2] but with a greater tendency toward parasitism.

The observation was made that wounds on the roots of black oaks (in particular, *Quercus velutina* Lam.) produced by the tramping of farm animals never heal when infected with the above fungus, but result eventually either in the death of the entire root or a part of it. Wounds not infected usually healed in a comparatively short time. These, among other observations, led to a supposition that this fungus was a slow parasite and hence to the beginning of experimental work.

Inoculations were made in healthy surface roots, inserting a wedge-shaped piece of bark and wood infected with the fungus into a corresponding cut in the healthy surface of a root. Before inoculation, the selected area as well as the piece to be inserted were washed in a solution of mercuric chloride and the wound afterward bound about with a cloth that had been steeped in a boiling mixture of tallow, beeswax, and rosin. This formed an impervious covering for the wound and was very durable. Ten different inoculations in all were made, five on *Quercus velutina* and five on *Fagus americana*. The first inoculations were all made in the fall of the year (1903) and were examined the following summer, and again in 1911.

Four inoculations on beech and three on oak were successful. The infections in beech by the following spring had extended outward from 2 to 3 inches from the wound in a very uniform manner. The bark and last annual ring only were attacked after the first eight months. The infection eventually progressed from 5 to 6 inches on either side of the original wound. By the spring of 1911 the infections had extended along the free

[1] Shear, C. L., N. E. Stevens, and R. J. Tiller. *Endothia parasitica* and related species. U. S. Dept. Agr. Bul. 380. 1917.

[2] Clinton, G. P. Chestnut bark disease, *Endothia gyrosa* var. *parasitica* (Murr.) Clint. Conn. Agr. Exp. Sta. Ann. Rpt. 1911–12: 359–453. 1913.

FIG. 1. Inoculation of *Endothia gyrosa* on living roots of *Quercus velutina*. A.—Point of inoculation. Infection spread to adjoining tree by means of grafted roots. Fungus visible at B and C.

upper surface of the roots of the oaks to the point where they became wholly covered with soil, an average distance of four feet. The fungus does not seem to have the ability of extending any appreciable distance along roots under the ground. The successful inoculations on the roots of the beech had extended but very little farther than those noted in 1903. In one case, where the root of the oak was small and free from the ground, girdling resulted, causing the free end of the root to succumb. In the other cases, only the free upper surfaces of the roots were killed. The yellow stromata made their appearance on either side of the cloth shield and gradually developed farther away from the original wound as the infections spread. In the spring of 1922 the inoculated roots were again examined. Of the four inoculations on beech, two infections had extended an average of one foot each way from the original point of inoculation. One had made but little progress and one had apparently died out. The infections were confined to this upper side of the roots. In the case of the infected oak, the fungus had become established in one instance on neighboring roots of another tree of the same species by means of grafted laterals (Fig. 1), the entire progress being an average of two feet. The progress of the remaining infections on oak was in like proportion, but was confined to the original root.

The inoculations were controlled by making exactly similar wounds in healthy roots and under exactly similar conditions. These wounds healed entirely with no apparent injury to the roots.

The fungus is by no means confined to the bark. The mycelium is fine, but by proper manipulation can be seen in the outer woody tissues, particularly in the larger cell elements. The mycelium in the woody portion of the root is confined principally to the last two or three annual rings. The chemical influence of the fungus extends much deeper and has a peculiar discoloring effect both on the bark and wood.

The fact that this fungus in artificially inoculated roots is able to gradually increase the infected area through the activities of its own mycelium, resulting in the death of the root or that part of it above ground, indicates the slow parasitism of this fungus. It enters entirely through wounds and will sometimes continue indefinitely producing its stromata on a very small infected area. This indicates the inability of the fungus to become parasitic during the early stages of growth. With a cumulative vegetative growth it may gradually acquire greater parasitic tendencies. The fungus may produce its stromata directly on a woody surface. They are usually, however, developed on the outer surface of the bark.

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NOTES ON SPOROPHORES OF POLYPORUS SCHWEINITZII FR. ON YELLOW PINE IN CALIFORNIA

LAKE S. GILL

The heartwood of living yellow pine (*Pinus ponderosa*) is often affected with the brown carbonizing rot usually attributed to *Polyporus schweinitzii*, although in California positive identification is not generally possible owing to the fact that fruiting bodies are rarely found in conjunction with the decay. It is true that sporophores of heartwood-destroying fungi are far less plentiful in the semiarid climate prevailing in California pine forests than in the fog belt of the coast or in the Pacific Northwest. The fairly common occurrence of sporophores on Douglas fir in the same forest type, however, seems to indicate that the inhibition in yellow pine is governed by factors inherent in the host, rather than by climatic conditions.

The ample collections of the Office of Forest Pathology, at San Francisco, made in pathological reconnaissance and field studies on the California pine forests since 1910, contain only one sporophore of *P. schweinitzii* known with certainty to have been growing on living yellow pine. This had been found near Norfork, Madera County, at an elevation of about 2,600 feet. It was noted as occurring on the base of a living tree and, judging by some charred bark scales and resin adhering to the back of the specimen, had been issuing from a fire scar. Another sporophore of the fungus taken from a scar at the base of what was either a yellow or Jeffrey pine standing in the same locality mentioned above, and a third collected near Beckwith, Plumas County, at an elevation of 5,500 feet, from the inside of a yellow pine stump which had been hollowed out by fire, are the only other records of immediate interest in the collection. It may be significant that all three sporophores were connected with wounds.

Two additional cases of *P. schweinitzii* fruiting on living *Pinus ponderosa* in the pine forests of the Sierra Nevada were observed during the summer of 1924. In one instance sporophores of the stipitate type with a large round pileus, such as are commonly seen growing from surface roots of Douglas fir, were found at a distance of five feet from the base of a yellow pine 45 inches in diameter breast high. There were no fruiting bodies on the bole itself. When this tree was felled, a continuous column of decay was found to extend through the bole for over 35 feet, rendering the first two 16-foot saw logs commercially worthless and necessitating a

deduction from the scale of the third. In general, cull due to *P. schweinitzii* is confined to the first log. The observation was made early in August near the Cow Creek Ranger Station, Tuolumne County, at an elevation of about 5,000 feet.

Some 30 miles south of this point, at an elevation of 5,400 feet along the road leading from Crane Flat into Yosemite Valley, a more unusual case was noted on May 30, on a yellow pine 40 inches in diameter at breast height. Not only were sporophores coming from surface roots, but 12 or 14 more were growing out from a healed fire scar which extended from the ground to a height of about 15 feet. These were most numerous in the lower part of the wound but were distributed at uneven intervals along its entire length. In size they varied from abortive knobs two or three centimeters in diameter to imbricate clumps of shelves 15 cm. high, 25 cm. wide, and 13 cm. deep. The irregular and convoluted character suggested impeded growth.

The evidence at hand lends further support to the presumption that in California the fungus is responsible for considerable decay in yellow pine. Apparently, however, it fruits sparingly on this species and produces sporophores on the bole only where an injury by fire or other agency permits the mycelium to grow out from the heartwood to the exterior of the tree. It is not impossible that the common form with the central stipe arising from surface roots also depends upon the presence of wounds for its development.

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PHYTOPATHOLOGICAL NOTES

Preliminary environmental studies on the take-all disease of wheat caused by Ophiobolus graminis sacc. The take-all disease has caused considerable damage to the wheat crop in foreign countries for many years and comparatively recently the disease has been found to occur in the United States. Although the take-all infested areas in this country are relatively small, it is important to note that as infection centers they are located at rather strategic points. The disease seems to have a firm hold in the center of the hard winter wheat belt, and observations show that it is steadily spreading.

Rather early in the investigations on take-all it was observed that the disease is more severe in certain districts than in others and also that in the same fields it is more pronounced some seasons than others. In connection with these observations on the development of the disease, irregularities in the fruiting habit of the parasite were also noted. Soil temperature and moisture are both important variables, and preliminary studies on the disease show that these factors influence its development to a considerable extent.

These studies were carried out in the greenhouse in Wisconsin soil temperature tanks. Owing to the cultural difficulties encountered when attempting to grow winter wheat under greenhouse conditions, it was not practicable to conduct the experiments over the entire life-history of the host, and accordingly all of the studies were confined to the early growth period. In all cases the experimental soil was inoculated with pure cultures of single spore strains of *Ophiobolus graminis*. Soil temperatures were maintained at intervals of approximately 4° C. between the extremes of 8° and 32° C. Soil moistures were varied simultaneously with temperature. They were based on the water-holding capacity of the soil and were maintained near 33, 54, and 71 per cent.

The results of four experiments show that under favorable soil conditions *Ophiobolus graminis* is an unusually vigorous root and tiller parasite. Although the fungus infected the host at all of the soil temperatures employed, the greatest injury occurred at unusually low soil temperatures (12°–16° C.). The injury was greatest at 12° C. in soil of the medium water content, whereas 16° C. produced the most severe injury in soils containing the least and the greatest amounts of moisture. Although the amount of injury was not great at 8° C., it was more severe than at 32° C. Diseased plants were rarely found in soil held near 32° C. At favorable

soil temperatures infection occurred at all soil moistures, but the greatest amount of disease occurred in soils containing the highest percentages of water.

The results obtained with soil temperature are of interest when compared with those obtained in the similar studies on the Helminthosporium foot-rot. In the case of both parasites, the greatest vegetative development occurs near 24° C., whereas Helminthosporium foot-rot is greatest at soil temperatures near 28°–32° C., and take-all is most severe at 12°–16° C. In the studies on the Helminthosporium foot-rot,[1] the senior writer suggested that severe infection at the high temperatures might be explained by severe weakening of the host, due to unfavorable temperature conditions. Obviously this explanation will not hold for take-all, since under normal conditions thrifty, robust plants develop at 12°–16° C. It appears therefore that the explanations for these results must be sought through a study of factors probably relating both to the parasite and the host which are more basic than the phenomena of growth rate and vigor as revealed by ordinary methods of weight and measurement.

The details of these environmental studies will be published later.—H.H. MCKINNEY and R. J. DAVIS, Office of Cereal Investigations, U. S. Department of Agriculture, and Wisconsin Agricultural Experiment Station.

A Mosaic on Winter Wheat and Winter Rye. This disease on wheat has been reported previously by the writer and his collaborators in papers dealing with wheat rosette, but until recently the malady was not definitely known to be a transmissible mosaic. This disease seems to possess all of the symptoms associated with the mosaics of other grasses. It has been observed on wheat in the rosette-disease areas since 1920. In the spring of 1925, it was observed on winter rye growing in infested soil at Granite City, Illinois, and in the same soil which had been transported to Madison, Wisconsin, for experimental study. What appears to be the same mosaic was also found by Dr. A. G. Johnson in winter rye growing as a cover crop in the orchards of the U. S. Department of Agriculture, Arlington Farm, Virginia. Microscopic examinations of mosaiced rye plants from all these sources reveal the presence of cell inclusions which are very similar to, if not identical with, those associated with wheat mosaic.

Experiments have shown that the causal agent of this mosaic persists in the soil year after year. Susceptible varieties of winter wheat never have failed to develop the disease when grown in infested soil out of doors. By manipulating environmental conditions in the greenhouse, it has been possible to transmit this mosaic from diseased to healthy plants by means

[1] Jour. Agr. Res. 26: 195–218. 1923.

of the expressed juice and ground tissue. Five experiments have been carried out with Currell and Harvest Queen varieties of wheat. In every case the disease developed in some of the inoculated plants. A total of 160 Currell plants were inoculated and 17 of these developed mosaic and cell inclusions. One hundred and twenty-four Harvest Queen plants were inoculated. Twelve of these developed mosaic and cell inclusions.

It is of special interest to note that all but one of the Harvest Queen plants affected with mosaic became quite dwarfed. They also developed a deep green color. In fact, these plants presented an appearance which seemed identical with that of plants affected with wheat rosette. This condition has never been found to occur in the Currell variety. In no case did mosaic or the rosetted condition develop in the uninoculated plants used as controls, and no cell inclusions were found in any of the control plants examined.

While the percentage of inoculated plants which developed mosaic is rather small, this is not surprising as other grass mosaics have not been transmitted readily by means of the expressed plant juice. The fact that one mosaiced Harvest Queen plant did not develop the rosette condition may be explained on a genetic basis.

Observations made over several years show that some varieties of winter wheat which have been bred pure for agronomic and botanical characters may produce plants, when grown in uniformly infested soil, which differ widely in their susceptibility to dwarfing (rosette) and in their ability to produce certain types of mosaic mottling.

Preliminary results from head selection studies suggest that types of mosaic may be determined in part by inheritable factors which are not necessarily homozygous in a so-called pure line variety.

As yet there is no evidence indicating that wheat mosaic is seed borne.

The relationship between this mosaic and other mosaics has not been determined.—H. H. MCKINNEY, Bureau of Plant Industry, U. S. Department of Agriculture.

Aplanobacter insidiosum n. sp., the cause of an alfalfa disease. In a recent number of PHYTOPATHOLOGY there is a brief description by Dr. F. R. Jones[1] of a new vascular, bacterial disease of alfalfa. Since that note was written, Dr. Jones has proved even more conclusively that this disease, which is unlike any previously described on alfalfa, is caused by bacteria.

[1] Jones, Fred Reuel. A new bacterial disease of alfalfa. PHYTOPATH. 15: 243–244. 1925.

The writer was asked to determine the cultural and morphological characters of the organism and the present note is preliminary to a joint paper now in preparation by Dr. Jones and the writer.

This new plant pathogene is briefly characterized as follows:

Aplanobacter insidiosum n. sp.

A short, non-motile rod,[2] measuring $0.7 - 1.0 \times 0.4 - 0.5\mu$ for single, and $1.8 - 2.0 \times 0.4 - 0.5\mu$ for paired rods. Capsules are formed on most media; no spores; aerobic; Gram positive; not acid fast.

Growth on culture media is very slow and never becomes heavy. Beef agar poured plates require 4–5 days and often longer for the colonies to reach a visible size. The growth is white at first, becoming pale yellow. Colonies on beef agar are circular; margins entire; surfaces smooth and shining; flat to slightly convex; usually uniform in structure and viscid in consistency. Media containing sugar produces a better growth than ordinary media, but in such media the yellow color of the growth is likely to become obscured partly or entirely by the formation in the growth of *blue* granules which impart blue, green, and violet colors to the bacterial growth. The medium is not stained. The growth on potato cylinders is also subject to this discoloration. Beef gelatin is slowly liquefied; Loeffler's blood serum is not liquefied. Nitrates are not reduced. No gas is formed. No indol or hydrogen sulphide is produced. Small amounts of acid are formed from dextrose, lactose, saccharose, and glycerine. There is a long delayed (16–20 days) curdling of milk. There is no growth in Cohn's solution. In Fermi's and in Uschinsky's solutions an occasional culture has clouded, but mostly there is no trace of growth. In spite of its slow growth the organism has considerable vitality and survives rather long exposures to sunlight, to freezing, and to desiccation.

The maximum temperature for growth is 31° C., the minimum temperature is below 1° C., and the optimum about 23° C. Its thermal death point is about 51–52° C. According to the descriptive chart of 1920 of the Society of American Bacteriologists, its group number is 5331–31135–1222.

The specific name, *insidiosum*, selected for this organism, is expressive of its rather slow but persistent progress in the host which is often seriously infected before there is any definite outward symptom of disease.—LUCIA MCCULLOCH, Bureau of Plant Industry, Washington, D. C.

[2] No, or very doubtful, motility has been discovered, and the many attempts to demonstrate flagella have been unsuccessful.